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Printed in Canada by: The Runge Press Limited, Ottawa

Imprimé au Canada par: The Runge Press Limited, Ottawa

Design: Ludvic Saleh & Associates, Ottawa

Conception graphique: Ludvic Saleh & Associates, Ottawa

Publié sans interruption depuis 1901, le *Journal canadien des sciences halieutiques et aquatiques* a paru sous plusieurs titres : *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937 et *Journal de l'office des recherches sur les pêcheries du Canada* 1938–1979.

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Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 46, No. 7, July 1989 **Volume 45, nº 7, juillet 1989**

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Patterns of Timing and Size of Wild Coho Salmon (*Oncorhynchus kisutch*) Smolts Migrating from the Keogh River Watershed on Northern Vancouver Island

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Irvine, J. R., and B. R. Ward. 1989. Patterns of timing and size of wild coho salmon (*Oncorhynchus kisutch*) smolts migrating from the Keogh River Watershed on northern Vancouver Island. Can. J. Fish. Aquat. Sci. 46: 1086–1094.

Coho salmon (*Oncorhynchus kisutch*) smolts leaving the Keogh River and its tributaries demonstrated consistent patterns in their timing and size. An initial early peak in the mean size of migrating smolts occurred several weeks before the peak in their timing, and in several years when smolt migration was monitored until July, a late peak in size occurred near the end of the smolt migration. Changes in the mean size of smolts were not related to their origin within the watershed, and varying age compositions were only partially responsible for the two peaks in mean size. Fish overwintering in tributary lakes grew faster than fish overwintering in tributary streams. A greater proportion of lake-origin smolts migrated to sea after only 1 yr in freshwater than stream-origin smolts. It appears that fish that wait until their second spring to smoltify are among the first to emigrate that spring. These relatively large 2+ smolts, combined with fast growing 1+ smolts that leave early during their first spring, cause the first peak in size. The small numbers of large smolts leaving the Keogh River at the ends of several of the smolt runs apparently left then because of minor flow increases in the river. As has been found with releases of coho smolts from nearby hatcheries, fish that left the Keogh River early in the smolt run generally had a more northerly marine catch distribution than later migrating smolts. A greater proportion of fish migrating to sea late in the smolt run were caught in the fishery than fish migrating early. Additional work examining the marine survival of early and late migrating wild smolts is recommended to enable detailed comparisons to be made between the optimal timing and size of wild and hatchery smolts.

Les tacons du saumon coho (*Oncorhynchus kisutch*) quittent le bassin de la Keogh selon un mode prévisible : plusieurs semaines avant les passages maximaux, la taille des poissons qui migrent est maximale et, quand les observations durent jusqu'en juillet, on observe un autre maximum de taille vers la fin de la migration. La taille n'est pas liée au lieu d'origine, et la composition démographique n'est que partiellement responsable des deux sommets de taille moyenne. Les poissons qui hivernent en lac grossissent plus vite que ceux qui hivernent en ruisseau, et une plus grande proportion des premiers que des seconds descend à la mer après un an seulement. Il semble que les poissons qui deviennent tacons au deuxième printemps sont parmi les premiers à émigrer cette année-là. Le premier passage de gros tacons est dû à ces poissons et aux tacons de première année qui ont grossi rapidement. Le petit nombre de gros tacons qui émigrent de la Keogh après plusieurs passages migratoires le font apparemment à cause de petits accroissements du débit de la rivière. Comme dans le cas des tacons de cohos relâchés de piscicultures voisines, les poissons qui quittent la Keogh tôt dans la saison sont généralement capturés plus au nord que ceux qui migrent plus tard. Les migrateurs tardifs sont pêchés en plus grande proportion que les migrateurs hâtifs. Il faudrait maintenant étudier la survie en mer des migrateurs hâtifs et tardifs afin de pouvoir comparer les données de migration et de grosseur relatives aux tacons sauvages et d'élevage.

Received October 11, 1988
Accepted March 9, 1989
(J9905)

Reçu le 11 octobre 1988
Accepté le 9 mars 1989

In their studies on the importance of various times and sizes at release for juvenile hatchery-raised coho salmon (*Oncorhynchus kisutch*), Bilton et al. (1982, 1984) hypothesized that it should be possible to manipulate release times and sizes to maximize growth and survival in the marine environment. If optimal release times and sizes do exist for hatchery stocks, it seems reasonable that natural selection pressures may have favoured stocks of wild coho with smolting tendencies similar to that observed for hatchery fish. Alternatively, other factors may control the timing and size of wild smolt migrations. However, there are few data on the migratory timing and sizes of wild coho smolts, and the impact of smolt size and timing on adult returns is poorly understood.

Salmonid populations have been investigated in the Keogh River on northern Vancouver Island (Fig. 1) since 1975 when a fish counting fence was installed to enumerate migrating adult steelhead (*O. mykiss*) (Ward and Slaney 1988). This 33-km long river drains an area of 129 km^2 and varies in flow between 0.1 and 254 $m^3 \cdot s^{-1}$. Hatchery outplants of coho to the Keogh have not been permitted although fish in the river have been impacted

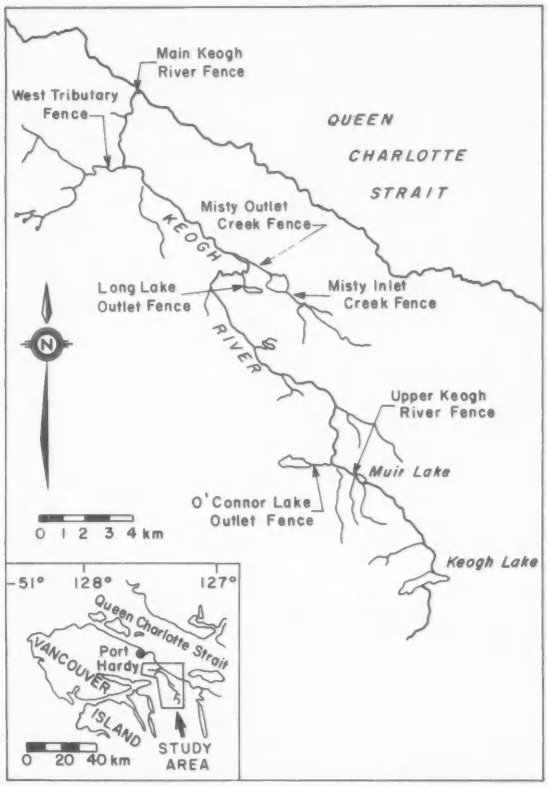

FIG. 1. Location of the fish counting fences in the Keogh River Watershed. Inset shows the location of the Keogh River Watershed on northern Vancouver Island.

by experimental stream fertilization (Perrin et al. 1987), instream enhancement structures (Ward and Slaney 1979, 1981), lake-pen rearing of steelhead smolts (Slaney and Harrower 1981), and logging. Between 55 000 and 105 000 coho salmon smolts leave the system annually. Preliminary analysis of data on the coho smolts leaving the Keogh River between 1979 and 1985 demonstrated that consistent trends in the timing and sizes of smolts existed. Therefore, we initiated a study to investigate if different overwintering environments were responsible for producing these trends. In this paper we discuss the patterns in size and timing for wild coho salmon smolts in the Keogh River in relation to their overwintering environment and age structure. Coded-wire tag data from commercial and sport fish catches are used to compare the marine distribution of fish that left the Keogh River early and later during the smolt run.

Methods

Coho salmon smolts were enumerated every spring between 1978 and 1987 at the main fish counting fence about 300 m upstream of the Keogh River mouth (Fig. 1). Smolt enumeration was only conducted during spring as earlier studies (e.g. Shapovalov and Taft 1954) indicated that most coho smolts migrate to the sea during spring. As well, smolt trapping in the Keogh River during the autumn and winter of 1975 and 1976 failed to capture any coho salmon smolts, although some coho parr were caught. During the enumeration of smolts leaving the Keogh River, the river was normally diverted away from the main fence via an overflow channel. Smolts were collected in trap boxes after passing over a large horizontal screen (expanded metal mesh, 9.9×22.2 mm). Occasionally, during freshets when it was not possible to divert all the river down the overflow channel, smolts were also collected using inclined-plane traps along the main fence (Mottram 1977). Trap efficiency tests were conducted usually twice per season. These tests consisted of capturing large (>120 mm) coho smolts in the trap boxes, clipping a corner of the caudal fin of 500 of these fish, and then releasing them about 50 m above the main fence. Subsequently, smolts captured in the trap boxes were examined for clipped fins. Trapping efficiency was usually about 90%.

Between 1977 and 1980, smolt traps were operated in various tributaries of the Keogh River as well as the upper mainstem river. These traps, similar in design to the main fence, are described by de Hrussoczy-Wirth (1979) and Smith and Slaney (1980). Lake-origin smolts were captured in 1977 and 1978 at the outlet of O'Connor Lake; predominantly stream-origin fish were captured in West Tributary in 1977 and 1978. Fish originating from a combination of lake and stream environments were captured in the upper Keogh River near the outlet of Muir Lake each year between 1977 and 1980 and in Misty Outlet Creek in 1977 (Fig. 1). To assist in distinguishing between differences in the timing and sizes of coho smolts that overwintered in lake and stream habitats, smolts were again trapped in 1986 at the outlet of O'Connor Lake, as well as at the outlet of Long Lake, and the inlet of Misty Lake (Fig. 1). There are no streams containing salmon upstream of O'Connor or Long Lake and no lakes upstream of the Misty Inlet Creek trapping site. The O'Connor Lake trap was operated continuously between 14 April and 27 May. The traps in Long Lake Outlet and Misty Inlet Creeks were operated intermittently between 20 March and 17 July, the number of 24-h periods per week depending upon the size of the smolt run and the availability of personnel. All traps were 100% efficient in capturing migrating smolts.

During the early part of the smolt migration, significant numbers of coho parr were often caught. These fish generally had parr marks and usually were smaller than the more silver smolts. Any coho less than 75 mm was considered a parr and all parr were excluded from the data analysis.

At each trap site, smolts were sampled randomly for fork length and weight; scales were taken from a subsample of these fish for subsequent age determination.

In 1977 and 1978, coho smolts captured at the main fence were coded-wire tagged (de Hrussoczy-Wirth 1979) and results were analyzed to assess the relationship between timing of smolt migration and catch in the commercial and sport fisheries. In 1977, three tag codes were used, corresponding to the early, peak, and late portions of the smolt migration. In 1978, fish migrating during the first half of the migration period had one tag code, while fish migrating during the second half of the migration had a separate code:

Year	Timing	Capture period	Approximate number tagged
1977	early	20 April–11 May	9 558
1977	middle	12 May–27 May	24 116
1977	late	28 May–21 June	7 862
1978	early–mid	30 April–13 May	9 489
1978	mid–late	13 May–21 June	18 856

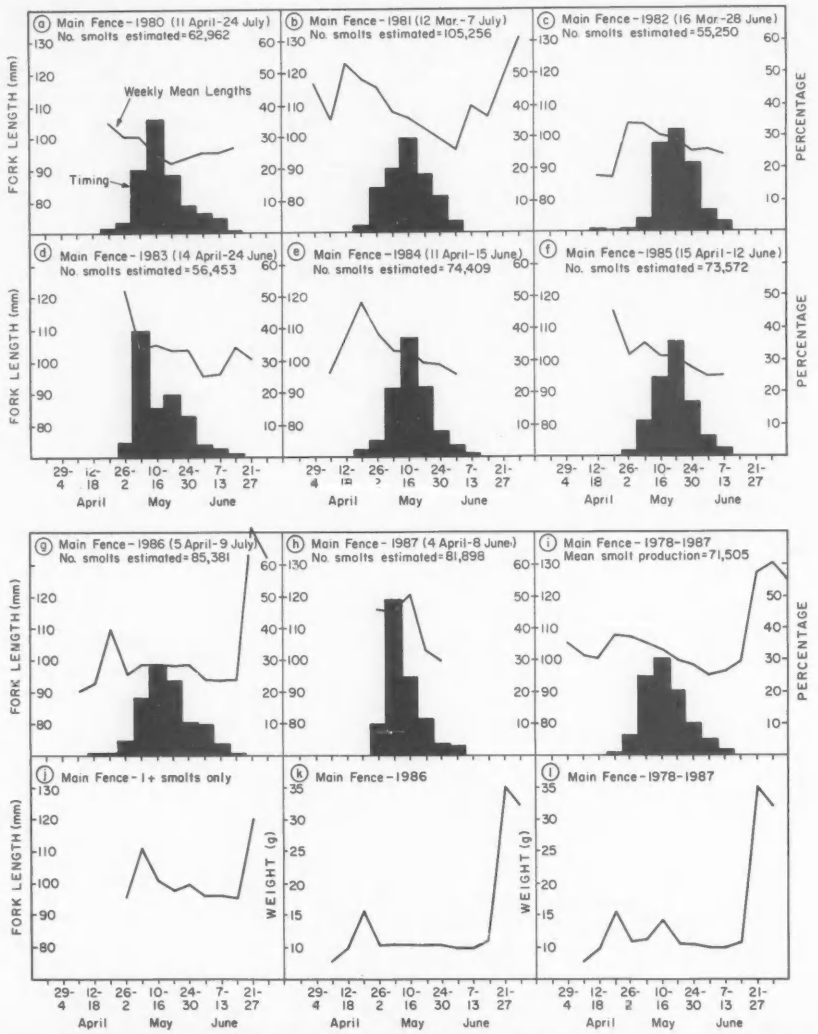
a Main Fence - 1980 (11 April-24 July)
No. smolts estimated = 62,962
Weekly Mean Lengths
Timing
b Main Fence - 1981 (12 Mar.-7 July)
No. smolts estimated = 105,256
c Main Fence - 1982 (16 Mar.-28 June)
No. smolts estimated = 55,250
d Main Fence - 1983 (14 April-24 June)
No. smolts estimated = 56,453
e Main Fence - 1984 (11 April-15 June)
No. smolts estimated = 74,409
f Main Fence - 1985 (15 April-12 June)
No. smolts estimated = 73,572
g Main Fence - 1986 (5 April-9 July)
No. smolts estimated = 85,381
h Main Fence - 1987 (4 April-8 June)
No. smolts estimated = 81,898
i Main Fence - 1978-1987
Mean smolt production = 71,505
j Main Fence - 1+ smolts only
k Main Fence - 1986
l Main Fence - 1978-1987
FORK LENGTH (mm)
PERCENTAGE
WEIGHT (g)
April
May
June

FIG. 2. Weekly mean lengths and timing of migration for coho smolts captured at the main fish counting fence on the Keogh River during 1980–87 (a–i), for 1+ smolts only (j), and weekly mean weights (k–l). The period the fence was operated each year is indicated in brackets. Sample size was always greater than 25 smolts measured each week; otherwise weekly means are not given. Weekly mean lengths in (i), and (j), and weights in (l) are not weighted by year.

The numbers of tagged fish captured in the commercial and sport fisheries in Alaska, British Columbia, Washington, and Oregon were obtained through the Mark Recovery Program. As commercial catches were sampled at a target intensity of 20% (Kuhn et al. 1988), the number of observed recoveries of tagged fish in the commercial fishery was multiplied by five. Approximately 25% of clipped coho caught by the sport fishery in the Strait of Georgia (body of water between Vancouver Island and mainland) in 1980–81 were reported (DPA 1982) so the number of reported recoveries in the sport catch was multiplied by four to obtain the estimated number in the sport catch.

Results

Size and Timing of Smolts at Main Fence

For each of the years for which adequate numbers of fish were measured throughout the smolt migration, there was a peak in the length distribution near the beginning of the run, usually in late April (Fig. 2 a–i). This was invariably followed by a decrease in the mean length of migrating smolts. In several of the years when smolt migration was monitored until late June (1981, 1983, and 1986), the mean length of migrating smolts again increased about this time. The same pattern was illustrated when the mean weights of migrating smolts were plotted by weekly intervals (Fig. 2 k–l).

The timing of the run was consistent. The peak migration period was usually in mid-May and never before 3 May or after 23 May. The first peak in mean smolt length was between 1 and 4 wk before the peak in timing (Fig. 2 a–i).

These consistent patterns in the size and timing of smolts at the main fence were examined by plotting length-frequency distributions by weekly intervals for each year. Results are

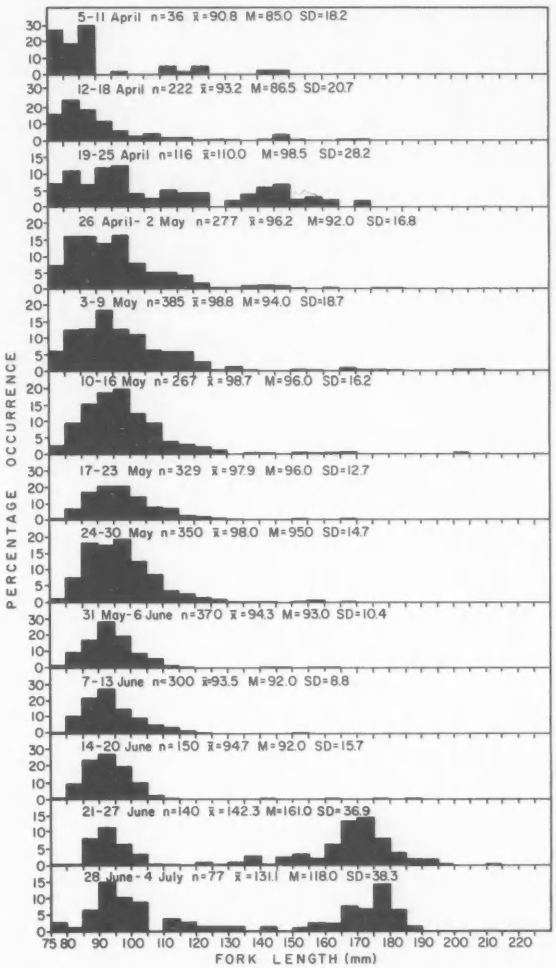

FIG. 3. Length-frequency distributions by weekly intervals for coho smolts captured at the main fish counting fence on the Keogh River during 1986. Mean ($\bar{x}$), median (M), and standard deviation (SD) are given for each weekly interval.

presented here for 1986, the year that the most intensive smolt sampling occurred (Fig. 3). In the early part of the run, the length–frequency distribution was skewed right because the smolts were predominantly small. As well, many of the coho leaving the river at this time were parr less than 75 mm which were excluded from the analysis.

The peak in the mean size of smolts during 19–25 April 1986 was at least partly attributable to the relatively high proportion of large 2+ smolts in the run. After mid-May, less than 10% of the smolts aged were 2+, while before 10 May, the proportion of 2+ smolts was generally much higher (Table 1).

As the run approached and then passed its peak in timing, the reduction in the proportion of 2+ smolts reduced the mean length of the smolts. However, the mean size of the younger cohort also declined and the frequency distribution approached normality. Late in the 1986 run (21 June–4 July), the frequency distribution was bimodal (Fig. 3). This resulted in an increased mean size of smolts. Interestingly, it appears that both modes were composed of predominantly 1+ fish (Table 1).

The weekly length-frequency distributions of the smolts at the main fence, and the age distributions of these smolts, partially explained the consistent patterns in mean smolt lengths. However, these patterns still existed when only lengths of 1+ smolts were examined (Fig. 2j). The possibility therefore existed that the timing and size of smolts migrating from different parts of the watershed were important in producing these patterns.

Size and Timing of Smolts Upstream of the Main Fence

There were no consistent differences in the timing of migration from various habitats (i.e. stream, lake, or stream/lake combination) (Fig. 4). The peaks in timing from the tributaries were about 1 wk before the peak at the main counting fence; in 1986, the peaks in timing from O'Connor Lake, Long Lake Outlet, and Misty Inlet Creek were during 3–9 May while the peak at the main fence was during 10–16 May. In most cases, a peak in the mean length of migrating smolts occurred before the peak of the smolt run timing.

Consistent differences existed in the size of smolts from the different habitats. In 1977, there were significant differences among the mean lengths of smolts leaving O'Connor Lake, the upper Keogh River, Misty Outlet Creek, and West Tributary ($p<0.01$; Kruskal-Wallis test). O'Connor Lake fish were significantly larger than the others ($p<0.01$), and West Tributary fish were smaller ($p<0.01$); differences among the lengths of fish leaving the upper Keogh River and Misty Outlet Creek were not significant ($p>0.05$) (nonparametric multiple comparisons using a STP test). In 1977, there were significant differences among the lengths of smolts from the four sites during the weeks between 26 April and 30 May ($p<0.05$; Friedman's test); i.e. the effect of time on smolt size was significant. In 1978, there were again significant differences among the mean lengths of smolts leaving the tributaries ($p<0.01$; Kruskal-Wallis test). Significant differences ($p<0.05$; STP test) existed between each group (O'Connor, upper Keogh River, and West Tributary). In 1978, there were no significant differences among the sizes of smolts leaving during the 5 wk between 26 April and 30 May. That year, 88% of the smolts leaving O'Connor Lake were 1+ (age determination sample size, $n=49$) 81% of the smolts leaving West Tributary were 1+ ($n=21$) and 96% of those captured in the upper Keogh River were 1+ ($n=55$) (de Hrussoczy-Wirth 1979). Therefore, the large size of smolts leaving O'Connor Lake was not a result of their age but rather their rapid growth. In 1986, there were significant differences among the sizes of smolts captured at O'Connor Lake, Misty Inlet Creek, and Long Lake Outlet ($p<0.01$; Kruskal-Wallis test). O'Connor Lake fish were significantly larger than fish from Misty Inlet Creek and Long Lake Outlet ($p<0.05$; STP test), but there were no significant differences among the sizes of fish from Misty Inlet Creek and Long Lake Outlet. The reason that the Misty Inlet Creek fish (stream origin) in 1986 were not smaller than the lake origin fish (Long Lake) was the high proportion of 2+ smolts leaving Misty Inlet Creek. Of 55 smolts aged from Misty Inlet Creek, 49% were 1+ ($\bar{x}$ length = 90.9 mm) and 51% were 2+ ($\bar{x}$ length = 101.4 mm). Of 122 smolts aged from Long Lake Outlet, 91% were 1+ ($\bar{x}$ length = 99.6 mm), and only 9% were 2+ ($\bar{x}$ length = 116.8 mm).

Relation between Timing of Smolt Migration and Catch

Fish tagged as smolts leaving the Keogh River contributed more to the commercial troll fishery than the commercial net

TABLE 1. Weekly mean lengths ($\bar{x}l$), standard deviations (SD), and sample sizes (n) indicative of proportions of 1+ and 2+ coho smolts captured at the main fish counting fence on the Keogh River.

		Year								
		1977			1978			1986		
Week	Age	n	$\bar{x}l$ (mm)	SD	n	$\bar{x}l$ (mm)	SD	n	$\bar{x}l$ (mm)	SD
19–25 April	1+							11	90.6	10.4
	2+							13	146.0	13.6
26 April – 2 May	1+	45	101.3	25.7				78	93.0	12.0
	2+	10	116.4	12.3				5	112.0	5.3
3–9 May	1+	65	116.4	16.5				24	95.8	13.3
	2+	14	127.1	21.1				4	115.0	36.5
10–16 May	1+	137	105.9		412	100.6		42	98.9	10.6
	2+	15	121.9		47	124.3		5	123.0	25.6
17–23 May	1+							50	97.8	8.9
	2+							3	118.3	11.1
24–30 May	1+	81	99.5	15.8	89	105.0	21.3	89	94.7	8.4
	2+	9	116.1	17.9	5	136.4	31.0	5	123.4	16.0
31 May – 6 June	1+	108	93.8	10.1	92	100.5	19.5	36	95.9	7.7
	2+	10	117.0	15.6	1	129.0		0		
7–13 June	1+	120	92.4		79	104.4	26.2	36	94.4	6.7
	2+	4	122.0		4	146.0	15.3	0		
14–20 June	1+	90	98.8	14.2	86	92.1	21.5	23	93.3	15.1
	2+	6	121.8	14.2	2	150.0	77.8	1	193.0	
21–27 June	1+							19	120.5	28.8
	2+							1	169.0	
28 June– 4 July	1+							4	141.0	36.8
	2+							0		

or sport fisheries (Table 2). With the exception of 10 fish originating from the mid–late group of smolts in 1978, all fish captured in the fishery were 3 yr old. The association between release timing and type of fishery (troll, net, or sport) was not significant (contingency table chi-square; $p>0.5$ for each year). However, a larger proportion of fish tagged as smolts migrating late in the smolt run was caught than of fish tagged early in the smolt run. An estimated 3.5% (333) of those smolts tagged leaving the Keogh River early in the smolt run of 1977 were captured, 3.4% (816) of the middle group were captured, and 5.5% (430) of the latest migrating fish were captured. Of those fish leaving the Keogh River in 1978, an estimated 6.3% (594) of the early group were captured, and 8.4% (1575) of the late group turned up in the commercial and sport catches.

The association between release timing in 1977 and 1978 and location of capture (Fig. 5) was significant (contingency table chi-square; $p<0.05$ for each year). A greater proportion of early migrating smolts was caught in northern areas than later migrating smolts. Late migrating smolts were caught more frequently in southern areas than earlier migrating smolts.

Discussion

Consistent patterns were found when 9 yr of coho smolt length data from the mainstem Keogh River were analyzed. There appeared to be two maxima in the mean length of smolts. The first was several weeks before the peak date for smolt output and a second maximum was found in late June in three of the years that smolt sampling extended this late in the season. The two peaks in mean size were only partially attributable to varying age compositions in the migrating smolts. It was found that 1+ smolts, which constituted approximately 90% of the total smolts, also exhibited a similar pattern of length and timing except that the first peak in mean size appeared to occur closer to the climax in numbers. In those years that a second size maximum was found, late-occurring minor flow increases may have been responsible for the small pulse of large smolts which caused this second maximum.

Smolts captured in tributaries to the Keogh River and the Keogh River near its headwaters also exhibited similar characteristics with large smolts leaving before the climax of the run. There were no significant differences in the timing of migration of smolts from various tributaries, but there were significant differences in the sizes of smolts from different locations. Smolts produced in O'Connor Lake were significantly larger than smolts originating from a combination of stream and lake environments (Misty Outlet Creek and upper Keogh River), while fish produced in the predominantly lotic environment of West Tributary were smallest. Lake-origin smolts from Long Lake were not bigger than stream-origin smolts from nearby Misty Inlet Creek, but this was due to the high proportion of 2+ smolts produced in Misty Inlet Creek. In a separate investigation, Swales et al. (1988) documented that underyearling coho in Misty Inlet Creek and Long Outlet Creek were significantly smaller than coho in Misty Lake and Long Lake. The overwintering location of smolts captured at the main enumeration fence on the Keogh River could not be identified by the size or timing of migrating smolts captured there.

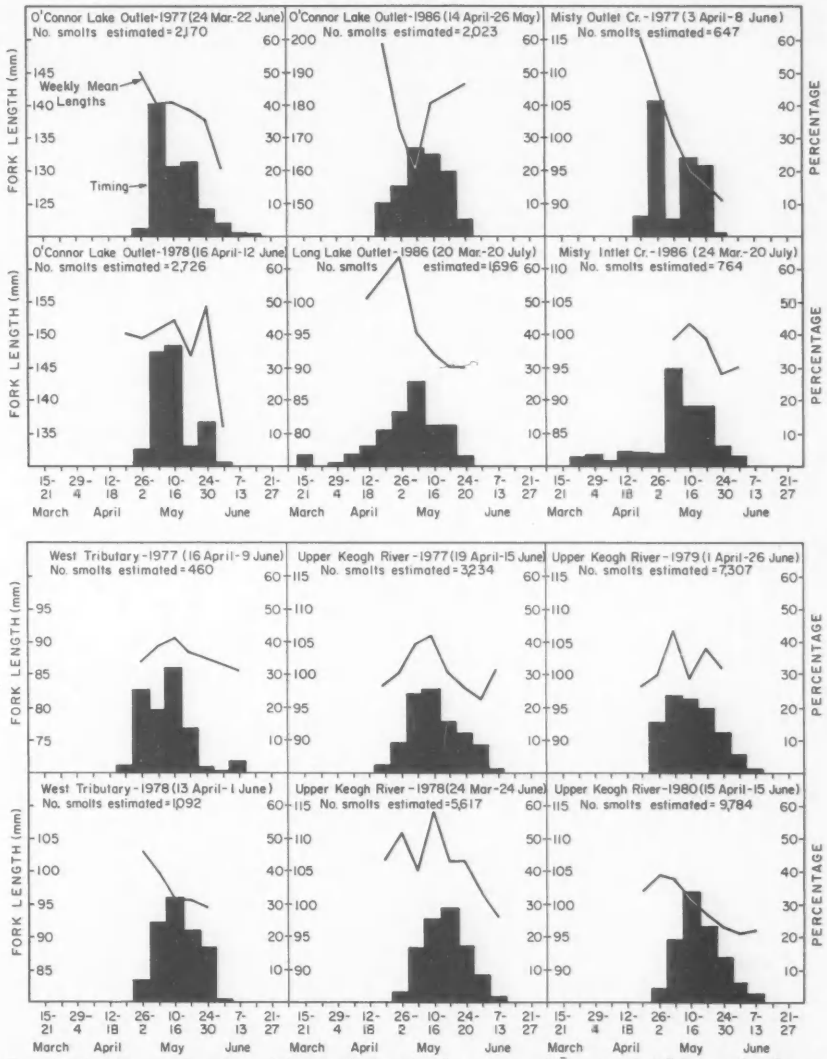
O'Connor Lake Outlet-1977 (24 Mar.-22 June)
No. smolts estimated = 2,170
O'Connor Lake Outlet-1986 (14 April-26 May)
No. smolts estimated = 2,023
Misty Outlet Cr.-1977 (3 April-8 June)
No. smolts estimated = 647
O'Connor Lake Outlet-1978 (16 April-12 June)
No. smolts estimated = 2,726
Long Lake Outlet-1986 (20 Mar.-20 July)
No. smolts estimated = 1,696
Misty Inlet Cr.-1986 (24 Mar.-20 July)
No. smolts estimated = 764
West Tributary-1977 (16 April-9 June)
No. smolts estimated = 460
Upper Keogh River-1977 (19 April-15 June)
No. smolts estimated = 3,234
Upper Keogh River-1979 (1 April-26 June)
No. smolts estimated = 7,307
West Tributary-1978 (13 April-1 June)
No. smolts estimated = 1,092
Upper Keogh River-1978 (24 Mar-24 June)
No. smolts estimated = 5,617
Upper Keogh River-1980 (15 April-15 June)
No. smolts estimated = 9,784
Weekly Mean Lengths
Timing
FORK LENGTH (mm)
PERCENTAGE
15-21 March
29-4 April
12-18 April
26-2 May
10-16 May
24-30 May
7-13 June
21-27 June

FIG. 4. Weekly mean lengths and timing of migration for coho smolts captured at fish counting fences operated in tributaries to the Keogh River and in the upper Keogh River. The period the fences were operated each year is indicated in brackets. Sample size was always greater than 15 smolts measured each week; otherwise weekly mean lengths are not given.

These paradigms in size and timing are not unique to coho leaving the Keogh River. For example, an initial peak in the mean size of steelhead smolts leaving the Keogh River about 10 May is followed by a relatively constant decline in mean size until late June when the size of smolts increases (B. R. Ward, unpubl. data). Steelhead smolts from the Keogh River average 70 mm longer than coho (Ward and Slaney 1988), and their peak timing is almost 2 wk later, occurring about 25 May. Weekly mean lengths of coho smolts leaving Carnation Creek on the west coast of Vancouver Island also varied consistently for many years, although the pattern was not as distinct as for coho leaving the Keogh River. Examination of 14 yr of data provided in Anderson (1983a, 1983b, and 1985) and Narver and Anderson (1974) revealed that for 1+ smolts, there was always an increase in mean smolt lengths until the end of April, although this increase sometimes continued until early June. In 8 yr there appeared to be a decrease in smolt size during the last half of May and June. Saunders (1967) found that older Atlantic salmon (*S. salar*) smolts (3+ and 4+) tended to migrate before young smolts. Ewing et al. (1984) reported that larger chinook salmon (*O. tshawytscha*) juveniles migrated before small ones and probably swam faster. These patterns of smolt size and migration timing have implications for the sampling of smolts in streams. We must be cautious when we compare lengths of smolts from different systems, particularly if the smolts are not all captured at the same time of year.

Apparently, similar pressures operate in different environments, which can produce the patterns of smolt size and migration timing that were found in the Keogh River. Folmar and Dickhoff (1980) stated that increasing photoperiod and water

TABLE 2. Estimated numbers (percentages in brackets) of coho salmon from the Keogh River that were captured in the troll, net, and sport fisheries. Smolts were coded-wire tagged in 1977 and 1978 according to their timing in the smolt migration.

	Release year				
	1977	1977	1977	1978	1978
Timing	early	middle	late	early–mid	mid–late
Troll fishery	261 (78.4)	603 (73.9)	307 (71.4)	471 (79.3)	1235 (78.4)
Net fishery	64 (19.2)	190 (23.3)	103 (24.0)	110 (18.5)	300 (19.1)
Sport fishery	8 (2.4)	23 (2.8)	20 (4.7)	13 (2.2)	40 (3.2)

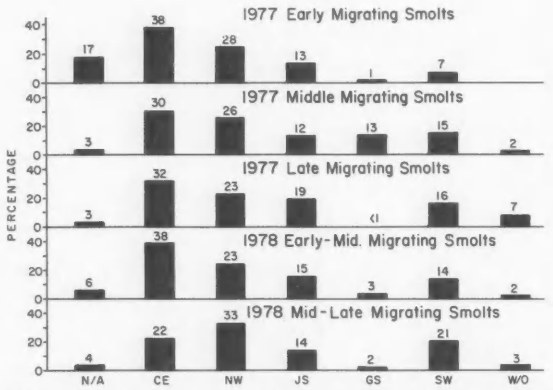

FIG. 5. Catch distributions of different groups of wild coho smolts migrating from the Keogh River during 1977 and 1978. N/A = northern (statistical areas 1–5) and Alaska; CE = central (areas 6–12 and 30); NW = northwest coast Vancouver Island (areas 25–27); JS = Johnstone Strait (areas 12 and 13); GS = Strait of Georgia (areas 13–18); SW = southwest coast Vancouver Island (areas 21, 23, and 24); W/O = Washington and Oregon.

temperature appeared to regulate the beginning and synchronization of smoltification and migration, but they also cited studies documenting the importance of an endogenous factor, manifested as critical size, which may be important in the timing of seaward migration. When fish, because of various environmental priming factors, are physiologically ready to smoltify, external releasing factors such as a heavy rain can trigger migration (Baggerman 1960; Grau 1981). When the lengths of 2+ smolts from the Keogh River were backcalculated to give their lengths 1 yr previously, these lengths were significantly less than those of 1+ smolts (J. R. Irvine, unpubl. data). These fish apparently had not reached their proper state of readiness to migrate during their first spring, but were generally among the first to emigrate the next spring.

It is of interest that the critical size for smoltification varies in different systems. We have shown how smolts produced in different environments in the Keogh River watershed emigrated at different sizes. Age 1 smolts in tributaries of the Cowichan River on Vancouver Island ranged between 74 and 100 mm (Argue et al. 1979). Drucker (1972) gives the average fork lengths of coho smolts for a variety of systems between Alaska and California. Average lengths of 1+ smolts ranged between 83 and 120 mm. The reason large 1+ smolts migrate before the peak of the outmigration is probably because, due to their large size, they are receptive to external releasing factors earlier than most smaller fish.

As mentioned earlier, the large smolts that left the Keogh River at the end of several of the runs may have left then because of minor freshet events in the river. These late-migrating large smolts, few in number, migrated well after the peak in migratory timing when many investigators have stopped enumerating smolts. In 1986, we maintained smolt enumeration fences in Long Lake Outlet and Misty Inlet Creek until early July, and large smolts were not captured in these tributaries late in the run. Flows were low in these tributaries in June and July. The large smolts captured at the main fence in June 1986 presumably originated from the main river, in which there was a small increase in flow in June. It has been shown that coho held in hatcheries can also smolt well after the peak in their normal run timing. Zaugg (1981) found that hatchery coho which had exhibited the silvery colouration and enzyme activity levels characteristic of smolts lost these traits after the normal smolt migration period. However, when these fish were released into the Columbia River in June or July, enzyme activity levels increased, silvery colouration returned, and the fish rapidly migrated to sea. As the large smolts that left the Keogh River in June were bigger than fish leaving in April or May, it does not seem possible that these fish could have waited until the following spring to emigrate. They apparently left in June because an increase in flow occurred then, and they presumably would have left later in the summer or fall if that is when the next postspring freshet occurred.

Although it was not possible in this study to compare smolts from different parts of the run in the returning adult escapement, it was possible to make comparisons in the commercial and sport fisheries. Fish originating from the Keogh River watershed were caught from Alaska to Oregon with most being caught by the commercial troll fishery near the North end of Vancouver Island. This gives us an indication of the extent of oceanic migration for coho salmon from the Keogh River. Some coho salmon spend their entire marine lives in "inside" waters (Milne 1957) while others migrate long distances offshore (Hartt and Dell 1986). The fact that a greater proportion of early migrating coho salmon smolts from the Keogh River were caught in northern areas than later migrating smolts implies that early migrating smolts may have different oceanic migration routes than later migrating smolts. Fish from early releases from the Quinsam Hatchery also had a more extended northward distribution than fish from later releases (Bilton et al. 1984; Morley et al. 1988). There is a need for further studies which focus on smolt run timing in relation to oceanic migration routes and timing.

We could not determine differences in natural mortality between early and late migrating smolts because adult escapement was not monitored when tagged fish returned to the Keogh River. A larger proportion of fish that had left the Keogh River after the peak in smolt timing were caught in the fishery than earlier migrating fish. This may indicate increased marine survival to the time of the fishery by late-migrating smolts, or it may simply reflect a difference in migratory routing and/or timing which affected the susceptibility of different groups of fish to fishing pressure.

The peak in timing of coho smolts leaving the Keogh River was earlier than the optimal release times recommended by Bilton et al. (1982, 1984) and Morley et al. (1988) for hatchery-

raised coho smolts. In the Keogh, the peak in timing was mid-May. Although natural smolt migrations occur later in more northerly systems (Drucker 1972), May timing peaks have been reported for other Vancouver Island populations (Anderson 1983a; Argue et al. 1979). Bilton and his colleagues examined the influence of time and size at release of coho from two hatcheries on the east coast of Vancouver Island. Results of experiments at Rosewall Creek Hatchery suggested that maximum returns (escapement plus catch) would be obtained by releasing 25.1-g juveniles on 22 June (Bilton et al. 1982). Coho smolts leaving the Keogh River in late June averaged 35 g. When Green and Macdonald (1987) analysed the data provided by Bilton et al. (1982), maximum returns were predicted by the late release of small, rather than large individuals. Results from a more northerly hatchery, the Quinsam, were obtained from releases made in 3 yr. Results from the first year (1980) suggested that maximum adult returns would be obtained from the release of 15.7-g juveniles on 4 June although size at release was less important than time (Bilton et al. 1984). Results from releases made in 1981 and 1983 confirmed that a tight "time window" for release centered at about 5 June, and that size at release was much less important than time (Morley et al. 1988). The release of smaller juveniles (14–30 g) was recommended, chiefly to reduce the incidence of jacking associated with large release sizes. Coho released from the Quinsam Hatchery migrated very quickly downstream to the estuary (Macdonald et al. 1987).

Although it is difficult to conclude from hatchery experiments what the optimal size at release of hatchery fish should be, it is of interest that most wild coho smolts that leave the Keogh River are much smaller than those released from hatcheries. Only during their two peaks in size were Keogh River smolts within the size range common for hatchery smolt releases. During the peak of their migration, Keogh River smolts averaged only 10.7 g. The importance of size for wild coho smolts is not understood.

Most wild smolts do not migrate at what appears to be the optimal time for survival in the ocean of hatchery fish, perhaps because the external releasing factors that initiate wild smolt migrations from freshwater occur earlier. Alternatively, downstream migration may be mainly passive and associated with changing stream flows. Perhaps if wild smolts were to delay their migration timing, they would fare better in the marine environment than they currently do, but instream survival would be poor. It is equally likely that late releases of hatchery coho do best because they do not have to compete with many wild smolts, and also perhaps because marine predators have not evolved to capitalize on the late arrival of these smolts. Further work examining the marine survival of early and late migrating wild smolts is underway to enable more detailed comparisons to be made between the optimal timing and size of wild and hatchery coho salmon smolts.

Acknowledgements

We thank all those who operated the smolt enumeration fences. We are indebted to A. R. Facchin for his help with computer analysis. Pat A. Slaney has managed provincial government research in the Keogh River watershed since 1975, and we are particularly grateful to him.

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Cultivation of *Pseudoterranova decipiens* (Sealworm) from Third-Stage Larvae to Egg-Laying Adults in Vitro

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Likely, C. G., and M. D. B. Burt. 1989. Cultivation of *Pseudoterranova decipiens* (sealworm) from third-stage larvae to egg-laying adults in vitro. Can. J. Fish. Aquat. Sci. 46: 1095–1096.

Third-stage larvae (L_3) of *Pseudoterranova decipiens* from cod (*Gadus morhua*) developed to maturity at 35°C in a two-step roller culture system. The medium was API-1 for step 1, and API-1 with bovine haem supplement for step 2. The gas phase was 85% N: 10% CO_2:5% O_2.

Le plein développement du troisième stade larvaire (L_3) de *Pseudoterranova decipiens*, parasite de la morue (*Gadus morhua*), a été obtenu selon la technique de Gey, dans des tambours tournants, et en deux étapes, à une température de 35°C. Le milieu de culture choisi pour la première étape était de l'API-1, substance à laquelle on a ajouté du hème bovin pour la deuxième étape. La phase gazeuse présentait le rapport N/CO_2/O_2 de 85, 10, et 5 %.

Received August 31, 1988
Accepted February 23, 1989
(J9865)

Reçu le 31 août 1988
Accepté le 23 février 1989

Pseudoterranova decipiens (Nematoda:Ascaridoida) lives as an adult in the stomach of seals and is commonly referred to as the sealworm. Seals become infected by eating fish which harbour the L_3 larval stage in their musculature. Although many different fishes carry the larval worms, their frequent occurrence in cod (*Gadus morhua*) previously caused them to be called codworm. The increasing abundance of this worm in the flesh of commercially important fishes in the Northwest Atlantic is a problem that costs the commercial fisheries, in Atlantic Canada alone, from $26.6 million (Malouf 1986) in 1984 to estimates ranging from $60–100 million.

Previous attempts to cultivate advanced stages of marine anisakine nematodes from the third stage larvae (L_3) from fish have had varying degrees of success: Townsley et al. (1963) were able to bring *P. decipiens* to maturity in vitro and observed eggs in the uteri of adult females but they do not mention the moult from the L_4 to adult nor do they mention release of eggs by gravid females; Van Banning (1971) and Grabda (1976) successfully cultured *Anisakis marina* (=*A. simplex*) to egg-producing adults in vitro; McClelland and Ronald (1974) cultured *P. decipiens* from freshly-hatched larvae to preadults but did not obtain fully mature adults; Carajal et al. (1981) cultured both *A. simplex* and *P. decipiens* to adults but do not mention presence of eggs; and finally, Douvres and Urban (1987), using a two-step roller culture system, were also able to culture *A. simplex* to egg-producing adults. Other workers have failed to replicate the results of Townsley et al. (1963).

The long-term objective of our present research program is a reduction in the abundance of the sealworm in commercially important fishes in Canadian marine waters. To this end, attempts are being made to examine the interaction in the stomach of seals between *P. decipiens* and a related anisakine, *Contracaecum osculatum*; this latter species has been suggested as being competitive with *P. decipiens* by Berland (1961) and McClelland et al. (1985). Using in vitro cultivation of both of these species, it was hoped that the nature of any interaction between them could be investigated.

Materials and Methods

Infective larvae (L_3) of *Pseudoterranova decipiens*, 2.0 to 2.5 cm long, were dissected out of the musculature of freshly-caught cod from Passamaquoddy Bay, N.B. Following several rinses with sterile 0.85% saline, they were washed in Earle's balanced salt solution containing 1 mL Gibco Antibiotic-Antimycotic/100 mL (20 mL Gibco Antibiotic-Antimycotic, Cat. No. 600-5245 AE, is prepared with: 10 000 units/mL penicillin G sodium; 10 000 µg/mL streptomycin sulfate; and 25 µg/mL amphotericin B as Fungizone). The larvae were cultivated in a two-step roller culture system as described by Douvres and Malakatis (1977). Groups of 25 larvae were placed in 500-mL glass roller bottles with 40 mL of API-1 at pH 4.5 and with a 85% N_2:10% CO_2:5% O_2 gas phase. After 7 d, larvae were transferred aseptically to clean, sterile, roller bottles with 80 mL API-1 supplemented with 48 µg bovine haeme. The second step was repeated every 7 d.

Eggs were recovered from the medium using a simple peptic digest solution (3 g pepsin, 3 mL 1 M HCl, made up to 100 mL with distilled water) and transferred to sea water at 5°C for 6 d. The eggs were then maintained at room temperature (~20°C) until they hatched.

Results and Discussion

Third-stage larvae of *Pseudoterranova decipiens* moulted to the L_4 between days 4–6 in vitro. This compares favourably with the moulting times, in vivo, of 48 h to 9 d, with 74.6% moulting between days 5–9, reported by McClelland (1980a). The shed L_3 cuticles of each nematode were found during the

first transfer of larvae to the haeme-supplemented medium. The L_4 were readily identified by the lack of a boring tooth and the presence of lips. A second moult occurred 12–14 d after the start of incubation. Nematodes emerging from this moult could be distinguished from the L_4 larvae by the presence of a prominent vulva in the females and characteristic flattening and recurvature of the tail with both pre- and postanal papillae in males. Whereas Townsley et al. (1963) observed a sudden increase in length of females, L_4 and young adults appeared to grow uniformly in our culture and growth rates of males and females were similar.

Females had begun to lay eggs by day 28 and by day 35 most of these were at the morula stage. A pair of adults was observed in copulo on day 38. By day 59, the adults had become lethargic, flaccid, and moribund. Males ranged from 3.1 to 7.5 cm and females from 4.5 to 10.5 cm long. These sizes are comparable with those of worms we have recovered from naturally infected harbour seals (males: 3.5–4.5 cm; females: 2.7–7.5 cm) and grey seals (males: 3.1–4.0 cm; females: 3.3–6.0 cm) and also to those reported by McClelland (1980b) for experimental infections in harbour seals (males: 3.74–5.4 cm; females: 4.15–6.08 cm) and grey seals (males: 3.73–6.44 cm; females; 4.17–8.21 cm). The larger, maximum sizes attained by the worms in vitro may be attributed to there being no loss of worms from the culture during the full growth phase whereas in vivo, worms may be passed from the stomach to the duodenum before reaching their potential maximum size.

Eggs laid by gravid females started to hatch after 6 d in sea water at about 20°C and ~30% had hatched by day 8. The newly-hatched L_2 showed a similar wriggling behaviour as L_2 from eggs of worms that matured in seals. Both the time taken to hatch and survival time of larvae in seawater at 20°C compare favourably with similar data from L_2 hatched from eggs at 20°C taken from gravid worms for a grey seal: hatching time, 6–8 d; larval survival, 5–6 d (C. G. Likely, unpubl. data). However, only about 30% of the eggs from females grown in vitro hatched compared with about 85% of the eggs from worms that matured in vivo.

Previous unpublished experiments in our laboratory indicated that the L_3 larvae of *P. decipiens* would moult readily to the L_4 and exsheath in any semi-defined medium, supplemented with calf serum and maintained at 35°C.

Bier (1976) reviews much of the in vitro work on anisakines, including *P. decipiens* and it is clear that the L_3 will moult to the L_4 in both defined and semi-defined media in which ecdysis also occurs. As reported by Davey and Kan (1968) the moult will also occur in 0.9% saline (at 35°C) but ecdysis does not occur. This moult occurred under atmospheric conditions, but in order for the L_4 to develop into sexually mature adults, both a complex medium (supplemented with haeme) and a 85% N_2:10% CO_2:5% O_2 gas phase is essential. Changes were observed in the condition of the medium during its use by *P. decipiens*.. The appearance changed from being slightly cloudy before use to flocculent after 7 d. In addition, the medium released a noticeable, pungent odour at transfer times, presumably the result of volatile ketones excreted by the worms as reported by Ackman (1976).

Acknowledgements

We are greatly indebted to Dr. F. W. Douvres for his generous advice on in vitro culture of anisakids. Bovine blood was kindly donated by Mr. Bud Pond of Chippin Bros., Fredericton, N.B. Fish (for L_3 larvae) were obtained through the Huntsman Marine Science Centre in St. Andrews, N.B. Mr. J. Tidswell provided help in establishing the incubator system. D. Campbell, A. Choudhury, L. Jarecka, B. M. MacKinnon, and N. Ramakrishna (Department of Biology, University of New Brunswick, Fredericton, N.B.), G. McClelland (Department of Fisheries and Oceans, Halifax, N.S.), and J. W. Smith (DAFS, Marine Lab., Aberdeen and Department of Fisheries and Oceans, St. John's, Nfld.) engaged in fruitful discussion with us. Financial assistance was provided by Natural Sciences and Engineering Research Council (operating grant A2358 to M.D.B. Burt) and through a contract from the Canada/New Brunswick Industrial Innovation and Technology Department Subsidiary Agreement.

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Effects of Condensed Tannins on Leaf Processing in Mid-Latitude and Tropical Streams: A Theoretical Approach

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Differences in the dynamics of allochthonous leaf processing in tropical streams are compared with those at mid-latitudes. Phytochemical differences are linked with transferral of energy in stream ecosystems. Condensed tannins, defensive secondary compounds that remain in leaves after cellular death, are suggested as inhibiting and altering leaf processing microorganisms. Comparisons between condensed tannin concentrations and leaf processing rates in mid-latitude and tropical streams support this view. Although many data are available for leaf processing rates per day in mid-latitude streams, there are few quantitative data on plant secondary compounds. The reverse is true for research in tropical regions. Leaf transfer experiments between Costa Rica and Michigan showed the interpretations based on processing rates per day may mask phytochemical differences in cross-biome studies. Processing rates normalized for temperature as degree days for such studies are preferred, as temperature differences then can be distinguished from biotic differences.

Les différences dans la dynamique de transformation des feuilles allochtones dans les cours d'eau tropicaux sont comparées à celles relevées aux latitudes moyennes. Les différences phytochimiques sont liées au transfert d'énergie dans les écosystèmes des cours d'eau. On croit que les tanins condensés, composés secondaires de défense qui demeurent dans les feuilles après la mort cellulaire, inhibent et altèrent les microorganismes de décomposition. Cette hypothèse est confirmée par la comparaison des concentrations de tanins condensés et des taux de décomposition des feuilles dans les rivières tropicales et aux latitudes moyennes. Bien que l'on possède une information détaillée sur les taux quotidiens de transformation des feuilles dans les rivières aux latitudes moyennes, on manque de données quantitatives sur les composés végétaux secondaires. L'inverse est vrai pour la recherche dans les régions tropicales. Les travaux comportant l'échange de feuilles entre Costa Rica et l'État du Michigan ont montré que les interprétations qui s'appuyaient sur les taux quotidiens de décomposition masquaient peut-être les différences phytochimiques relevées dans les études qui recoupent des biomes distincts. Pour ces études, on préfère normaliser les taux de transformation en fonction de la température sous la forme de degrés-jour, étant donné que les écarts de température peuvent alors être distingués des différences biotiques.

Received April 14, 1988
Accepted February 23, 1989
(J9666)

Reçu le 14 avril 1988
Accepté le 23 février 1989

In terrestrial communities, condensed tannins (proanthocyanidins) have been suggested as protecting leaves, bark, seeds, and root tissue from microbial invasion (Zucker 1983), as antibiotic agents (Porter 1986), and as defensive chemicals against herbivores (Swain 1978; Barbosa and Krischik 1987).

Condensed tannins in leaves that fall into streams also could affect aquatic bacterial and fungal activity, as they are not sequestered back into living plant tissue prior to leaf abscission. In this review I examine the hypothesis that leaves rich in condensed tannins falling into heterotrophic streams inhibit aquatic fungal and bacterial utilization of those leaves. By so doing, tannins alter biotic interchanges and energy transfers among the micro- and macroorganisms directly or indirectly dependent on the leaves. Leaves are a very important organic source for streams; any alterations to the utilization of leaves will affect the stream's energy budget.

Leaves that fall into streams draining watersheds low in available nutrients contain, on average, more condensed tannins than leaves produced on nutrient-rich soils (Janzen 1974). Higher levels of condensed tannins can be expected in leaves from tropical areas, dry forests, and boreal areas than in those from mid-latitude deciduous forests. The latter have been the sites for most of the research on the dynamics of leaf processing. Yet, the effect of condensed tannins on leaf processing is expected to be most profound in aquatic environments, where there has been little research on allochthonous inputs.

Data used to support the hypothesis come from a literature search of condensed tannin concentrations in leaves, from papers on processing rates of leaves in streams, and from transfer experiments on processing rates of leaves in Costa Rican and Michigan streams.

Background and Theory

Ecologists have been concerned with the utilization of leaf inputs in streams for nearly two decades, as those inputs are major organic resources for heterotrophic stream processes (Hynes and Kaushik 1969; Mathews and Kowalszewski 1969; Kaushik and Hynes 1971; Iverson 1973; Petersen and Cummins 1974; Triska et al. 1975; Suberkropp et al. 1976; Anderson et al. 1978). Phytochemical ecology of plant–animal interactions so richly studied of late in terrestrial systems (Feeny 1970; Har-

borne 1972; 1982; van Emden 1973; Gilbert and Raven 1974; Rhoades and Cates 1976; Swain 1977, 1978; Rosenthal and Janzen 1979; Macauley and Fox 1980; Fox 1981; Futuyma and Slatkin 1983) have not been explored for aquatic systems. Efforts at relating leaf litter utilization in streams to secondary plant compounds may further our understanding of cause-and-effect relationships between defensive plant compounds and trophic dynamics among aquatic organisms.

As condensed tannins are also found in bark (Stafford 1985), there is commercial value in studying the effects of condensed tannins on aquatic fungi and bacteria. Damage from logging may be diminished if the stripped bark of species known to be rich in tannins is not discarded in streams and rivers. Furthermore, streams draining areas with vegetation high in condensed tannins (as in certain tropical areas) may contain microorganisms adapted to condensed tannins as a carbon source. Microorganisms from those areas could be tested for their usefulness in wood processing.

Leaves of different plant species are processed in streams at different rates (Kaushik and Hynes 1971; Sedell et al. 1975; Short et al. 1980). These rates were modeled by Petersen and Cummins (1974) as the decay coefficient, $-k$, $\log (\%R_e/100)/t$, where $\%R$ is the percent of initial dry mass remaining, and t is the number of days in the stream. Decay coefficients ($-k$/d) were categorized by Petersen and Cummins (1974) as being (1) fast, >0.010, (2) intermediate, $0.0101 < -k < 0.005$, and (3) slow, 0.005 or less.

The observation that some leaves are more resistant than others led researchers to look at dynamics of leaf conditioning by aquatic microorganisms as a prelude to aquatic insect processing (Kaushik and Hynes 1968; Triska 1970; Bärlocher 1980). The invasion of the leaf by aquatic hyphomycetes conditions leaf surfaces for later bacterial colonization (Suberkropp and Klug 1976, 1981), which in turn facilitates and increases insect consumption rates (Triska 1970; MacKay and Kalff 1973; Petersen and Cummins 1974).

Physicochemical data have been related to leaf processing rates: carbon–nitrogen ratios (Witcamp 1966); percent lignin (Minderman 1968; Van Cleve 1974; Fogal and Cromack 1977); polyphenols and leaf hardness (King and Heath 1967); and actual evapotranspiration (Meentemeyer 1978). Many of the data have not been linked directly with rheophilic microbial and macrofaunal communities and most of the relational data from mid-latitude regions.

Condensed tannins have been implicated as antibiotic and antifungal agents (Harrison 1971; Grant 1976; Zucker 1983; Porter 1986), but aquatic hyphomycetes and bacteria enhance leaf quality for aquatic invertebrate and vertebrate consumers. Thus, if condensed tannins decrease leaf processing rates in streams, they should influence the ecology of the biotic community by decreasing consumption rates and by altering spatial and temporal food linkages among the primary and secondary consumers.

Mid-Latitude and Tropical Comparisons

Mid-latitude deciduous forest streams and tropical wet lowland forest streams differ in a number of ways (see Table 1). Stream water temperatures vary little seasonally in lowland tropical forests (Bishop 1973; Sioli 1975; Stout 1982; Day and Davies 1986) when compared with mid-latitude forested streams (Ward 1985). Differences in water temperatures can affect leaf processing rates (Witcamp 1966; Suberkropp 1984), especially for leaves that contain easily leached defensive compounds (such as water-soluble latex). These differences have to be considered when making latitudinal comparisons.

Rainfall in tropical wet lowland forests is less seasonal and usually higher, on an annual basis, than in mid-latitude deciduous forests (Minshall et al. 1983). Microbial activity should be less seasonal as well (Suberkropp 1984) and more constant, on an annual basis, in tropical steams, given the more constant and warmer air and water temperatures. Deterrents to microbial invasion by water-insoluble secondary compounds should be even more pronounced in those leaves.

In wet tropical forests, some mono- and dicotyledenous leaves remain on trees for several years while undergoing continual microbial, invertebrate, and vertebrate herbivory (Bentley 1979; Vandermeer et al. 1979). Plants rich in secondary compounds and/or physical barriers that are adapted to withstand continual herbivore pressure are likely to produce "expensive" (Janzen 1974), long-lived leaves. Any plant defensive compound that is not sequestered back into the plant prior to leaf drop and is not leached quickly after leaf drop could play a vital role in leaf processing in streams.

There is no leaf-fall period in tropical wet lowland forests comparable with the autumnal season of mid-latitude deciduous forests. Leaves fall throughout the year into the tropical streams and are always available to the aquatic biota. Some microorganisms, arthropods, and fish may specialize on those continual inputs in tropical areas. Leaves that lack chemical or physical deterrents and are processed rapidly are expected to be utilized by mobile consumers; slowly processed leaves are expected to be more important as physical substrata for stream biota that derive nutrition from seston and/or slowly released products of decomposition.

TABLE 1. Comparisons between mid-latitude deciduous forest streams and tropical wet lowland forest streams.

Characteristic	Mid-latitude	Tropical
Temperature fluctuations	strongly seasonal	weakly diurnal
Rainfall	seasonal, lower	less seasonal, higher
Microbial effect	seasonal	aseasonal*
Leaf longevity	spring–fall	up to 4 yr
Leaf inputs	seasonal	year-round
Leaf chemistry (percent dry mass)		
resins	lower	higher
alkaloids	lower	higher
condensed tannins	lower	higher
Array of leaf processing rates	normal distribution	bimodal distribution*

*Indicates hypothesized differences.

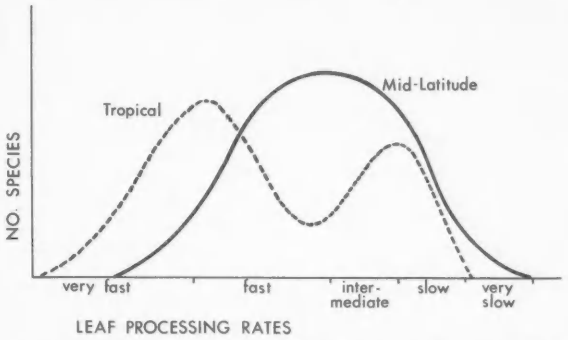

FIG. 1. Hypothesized comparisons between leaf processing rates in mid-latitude streams and in tropical streams. Solid line represents mid-latitude species. Dashed line represents tropical species.

It is in tropical environments that the highest diversity and richest concentrations of defensive plant compounds exist, and these include condensed and hydrolyzable tannins, alkaloids, resins, and gums (Janzen 1974; Gibbs 1974; Levin 1976; McKey et al. 1978; Rosenthal and Jansen 1979; Oates et al. 1980; Waterman et al. 1980; Arrhenius and Langenheim 1983; Coley 1983).

Condensed Tannins

Condensed tannins, a major class of defensive compounds, do not appear on most stream ecologists' "lists" of chemical compounds to be analyzed in allochthonous leaf studies except for Irons et al. (1988). Condensed tannins are suggested as protecting plant photosynthetic, seed, and root tissues by complexing themselves with plant pectins and celluloses. Those complexed products in the plants form barriers against pectinases and cellulases produced by many microorganisms as they try to penetrate plant tissue. Zucker (1983) suggests that condensed tannins have protective capabilities against microbial invasion but not against phytophagous invertebrates, but Swain (1978) believes that both microorganisms and invertebrates are deterred by condensed tannins in plants. Unlike hydrolyzable tannins, which Zucker (1983) thinks are the protective agents against invertebrates and vertebrates, condensed tannins are not sequestered back into the plant during leaf senescence. After leaf drop, intact condensed tannins would delay microbial processing. In tropical wet forests, delayed microbial decomposition could enhance recycling efficiency of leaf micro- and macronutrients by reducing their losses via leaching during heavy rains (Stark and Jordan 1978).

Condensed tannins are considered to have evolved prior to hydrolyzable tannins (Swain 1978). They are found in the horsetail (*Equisitum*), some ferns, gymnosperms, monocotyledons, and dicotyledons (Lawton 1976; Swain 1978), and they are the most widely distributed group of tannins in vascular plants (Crankshaw and Langenheim 1981). Hydrolyzable tannins are found only in dicotyledons. Concentrations of condensed tannins usually are higher in the more primitive plant families (Sporne 1973; Gibbs 1974). Primitive angiosperm families contain higher percentages of species with condensed tannins than do the more advanced families (Bate-Smith and Metcalfe 1957).

The evolution of flowering plants is thought to have occurred primarily in humid tropical areas, and it is there that the more primitive angiosperm families are found rather than in mid-latitude regions (Corner 1949; Harborne 1977; Raven and Axelrod 1981). Angiosperm leaves that fall into tropical streams are expected to contain, on average, more condensed tannins than such leaves falling into mid-latitude deciduous forest streams. Waterman (1983) states that more than 70% of rainforest tree species contain significant quantities of condensed tannins, hydrolyzable tannins, or both. Some tropical leaves contain more than 40% dry mass condensed tannins (Janzen and Waterman 1984). Plants rich in condensed tannins (that is, more than 5% dry mass) are more resistant to both terrestrial and aquatic microbial processing than are leaves containing no or low concentrations of condensed tannins. Tannins impart strong defenses against fast microbial processing and are an important addition to the list of physicochemical factors that presently are linked with slow decomposition rates such as lignin content and leaf toughness.

A hypothetical model (see Fig. 1) predicts that leaves lacking or low in condensed tannins are processed at fast to intermediate rates in mid-latitude streams; similar leaves would be processed very fast ($-k/d>0.05$) to fast ($0.01 < -k/d<0.05$) in tropical streams. It is predicted that leaves rich in condensed tannins are processed slowly to very slowly in mid-latitude streams and slowly in tropical streams. If leaves of those tropical species were transferred to mid-latitude forested streams, the rates would be even slower, especially because most leaf processing, and studies of the phenomenon, occur in fall and winter. Most leaf processing coefficients are expressed relative to days in stream, $-k/d$; whereas, leaf loss data used for latitudinal comparisons should be related to degree days to normalize water temperature differences.

Leaf Tannin Concentrations and Processing Rates in Mid-Latitude Streams

Mid-latitude leaves lacking or low in condensed tannins have fast ($-k/d>0.01$) processing rates (see Fig. 2A and Table 2); leaves with intermediate levels of condensed tannins are processed at an intermediate rate ($0.005< -k/d<0.01$) with one exception, *Liquidamber styracilua*. It is placed in the intermediate level for condensed tannins, although there is disagreement as to whether it is low (Bate-Smith 1962) or intermediate (Bate-Smith and Lerner 1954; Jay 1968). Leaves with slow processing rates ($-k/d<0.005$) are generally high in condensed tannins. Box elder (*Acer negundo*) is listed as having high levels of condensed tannins (Bate-Smith 1977), yet its leaves are processed quickly. A beech, *Nothofagus solandri* var. *cliffortoides*, has a very slow processing coefficient ($-k/d = 0.0010$ to 0.0025) (Rounick and Winterbourn 1983), but no quantitative condensed tannin data are available for its leaves. Davis and Winterbourn (1977) worked with this species and found very few aquatic hyphomycetes on leaves. They also noted a lack of hyphal penetration even after 4 mo incubation in a New Zealand stream. Based on their findings and the fact that the genus is a member of a primitive angiosperm family, that species is predicted to have high concentrations of condensed tannins. Cambie et al. (1961) listed only presence or absence for members of this genus; the species contained condensed tannins. Uprichard et al. (1975) show that both heartwood and sapwood of this beech contained abundant amounts of condensed tannins, but they did no leaf tissue analysis.

Of the 39 mid-latitude species for which condensed tannin data are available, 19 either lack or have very low condensed tannin concentrations (Fig. 2A). As a group, they are processed

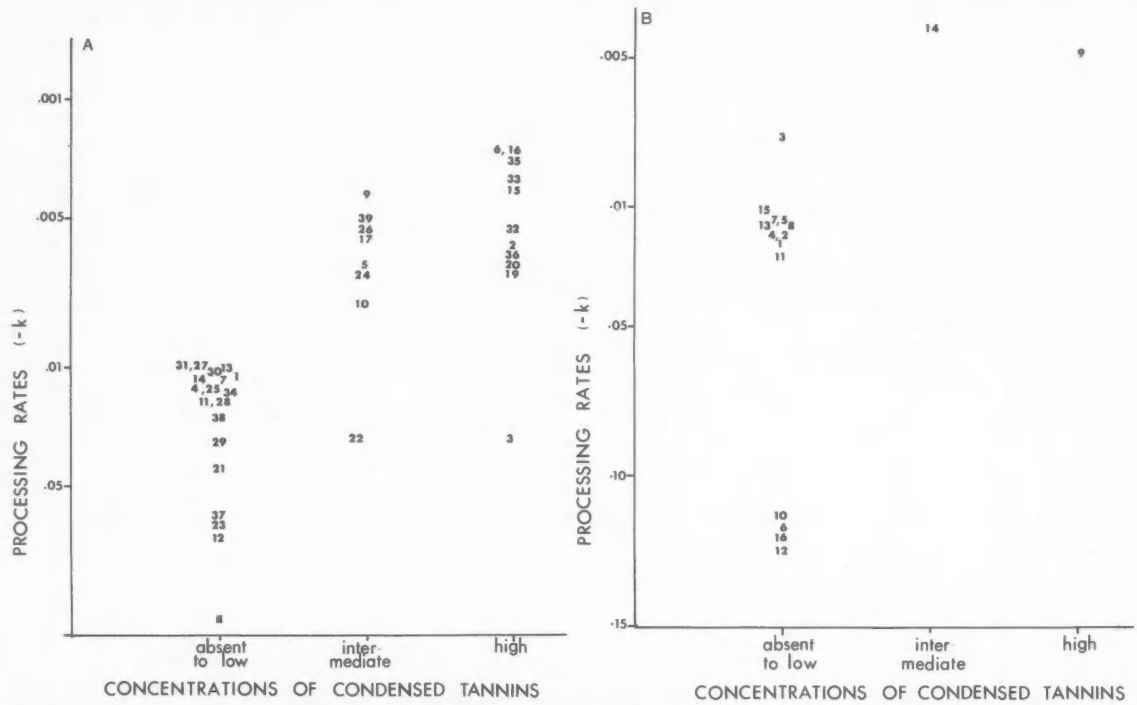

FIG. 2. (A) Mid-latitude leaf processing rates as a function of condensed tannin concentrations. (Processing rates from Webster and Benfield 1986.) (B) Tropical leaf processing rates as a function of condensed tannin concentrations.

fast (mean $-k$/d = 0.0312, SD = 0.0245). Eight species have intermediate condensed tannin levels, with an intermedite processing rate of 0.0094 (SD = 0.0096). If the species for which condensed tannin concentrations are open to question are excluded, the mean $-k$/d rate would be 0.0060 (SD = 0.0013). Twelve species have high condensed tannin levels. Without *Acer negundo*, which was included in Fig. 2A, the mean processing rate is slow (mean $-k$/d = 0.0045, SD = 0.0017).

The processing coefficient for a coniferous gymnosperm, the Douglas fir (*Pseudotsuga menziesii*), was 0.0055. It has the highest condensed tannin concentration by dry leaf mass for any mid-latitude species (20% according to Stafford (1985)), although it is lower than for many tropical angiosperms (see Table 4). Anderson and Grafius (1975) have shown that needles from *P. menziesii* require a long conditioning period. Although the toughness and waxiness of the needles are probably very influential, the high condensed tannin concentrations also should extend the conditioning period.

Three plant families, Accraceae, Betulaceae, and Salicaceae, include many species; as a group these species are highly variable with respect to condensed tannin concentrations (Bate-Smith and Lerner 1954; Binns et al. 1968; Jaggi and Haslam 1969; Bate-Smith 1975, 1977). Species in the Fagaceae are usually rich in condensed tannins, whereas those in the Oleaceae seldom contain tannins. Among species in these five families there is a gradation from no tannins to rich concentrations of condensed tannins. These families are well represented throughout the world, and many of their species have been used in leaf processing studies. All five families would be prime choices for research on the relationship between leaf processing dynamics and condensed tannin concentrations in similar sized streams at similar altitudes along latitudinal gradients.

Leaf Tannin Concentrations and Processing Rates in Tropical Streams

Published data for leaf processing rates in tropical streams are meager (see Fig. 2B and Table 3), although data for condensed tannin concentrations are more abundant for the tropics than for the mid-latitudes (see Table 4). There are processing coefficients for tropical leaves deposited on forest floors (for example, Ewel 1976; Tanner 1981; Proctor 1984; Spain and LeFeuvre 1987), but these coefficients cannot be confidently converted to rates in flowing waters.

Figure 2B and Table 3 include only the tropical species for which processing coefficients were determined for leaves in streams. Condensed tannin data for tropical species for which associated leaf processing data are lacking are presented in Table 4. Additional data may be found in Bate-Smith (1962), Bate-Smith and Metcalfe (1957), Bate-Smith and Lerner (1954), Gibbs (1974) and Hegnauer (1961–1964, 1966, 1969, 1973, and 1986). Condensed tannin concentrations for the species listed in Table 4 and in the above references were presented to identify tropical species with high concentrations, which thus are potential subjects for testing the hypothesis in this paper.

Of the 14 species low or lacking in condensed tannins, processing rates for four were very fast ($-k$/d mean = 0.1182, SD = 0.0050), for nine were fast (mean = 0.0178, SD = 0.0045), and for one, *Cordia borinquensis*, was intermediate ($-k$/d = 0.0077). The mean processing rate for these 13 "fast" species was 0.0487 (SD = 0.0484). The category with intermediate levels condensed tannins contains only one tropical species for which there are leaf processing data in an aquatic environment. That taxon, *Ardisia* sp., was processed slowly in a Costa Rican

TABLE 2. Mid-latitude plant species used in Fig. 2A, with $-k$/d values, condensed tanning levels, and references for condensed tannins (CT).

Species	Family	$-k$/d*	C.T.	References
1. *Acer circinatum*	Aceracae	.0136	Low	[a]
2. *A. macrophyllum*		.0059	High	[b]
3. *A. negundo*		.0345	High	[b]
4. *A. platanoides*		.0205	Low	[b]
5. *A. rubrum*		.0067	Intermediate	[b]
6. *A. saccharum*		.0029	High	[b]
7. *A. saccharinum*		.0150	Low	[b]
8. *Oplopanax horridum**	Araliaceae	.0960	Low	[a]
9. *Alnus glutinosa*	Betulaceae	.0043	Intermediate	[c]
10. *Betula papyrifera*		.0080	Intermediate	[d]
11. *Baccharis glutinosa**	Compositae	.0223	Low	[e]
12. *Tussilago farfara**		.0680	Low	[e]
13. *Cornus amomum*	Cornaceae	.0115	Low	[a,c]
14. *C. florida*		.0187	Low	[c]
15. *Rhododendron maximum**	Ericaceae	.0040	High	[c,e,f]
16. *Quercus macrocarpa*	Fagaceae	.0029	High	[c,g]
17. *Fagus sylvatica*		.0054	Intermediate	[c,e]
18. *Nothofagus solandri* var *cliffortiodes*		.0019	High?	[e,h]
19. *N. cunninghami**		.0068	High?	[h]
20. *N. fusca*		.0064	High?	[h]
21. *Nymphoides peltata**	Gentianaceae	.0459	Low	[e]
22. *Liquidambar styraciflua*	Hamamelidaceae	.0330	Intermediate	[c,i,j]
23. *Elodea canadensis*	Hydrocharitaceae	.0644	Low	[k]
24. *Juglans nigra**	Juglandaceae	.0070	Intermediate	[a,c,e]
25. *Umbellularia californica*	Lauraceae	.0201	Low	[c,e]
26. *Robinea pseudoacacia*	Leguminosae	.0053	Intermediate	[l]
27. *Decodon verticallatus*	Lythraceae	.0101	Low	[i]
28. *Liriodendron tulipifera*	Magnoliaceae	.0224	Low	[e, l]
29. *Potamogeton nodosus*	Najadaceae	.0360	Low	[k]
30. *Fraxinus americana*	Oleaceae	.0130	Low	[c, e, i]
31. *F. niger*		.0097	Low	[e]
32. *Pseudotsuga menziesii*	Pinaceae	.0055	High	[n]
33. *Platanus occidentalis*	Platanaceae	.0039	High	[j]
34. *Ranunculus calcareus**	Ranunculaceae	.0210	Low	[e, i]
35. *Salix viminalis*	Salicaceae	.0030	High	[o, p]
36. *S. alexensis*		.0063	High	[d]
37. *Ribes bracteosum**	Saxifragaceae	.0620	Low	[i]
38. *Tilia americana*	Tiliaceae	.0222	Low	[j, e]
39. *Ulmus americana**	Ulmaceae	.0051	Intermediate	[c, e]

*A related species analyzed for condensed tannins. Low indicates low or nonexistent.
[a]Gibbs (1974).
[b]Bate-Smith (1977).
[c]Bate-Smith and Lerner (1954).
[d]Irons et al. (1988).
[e]Bate-Smith and Metcalfe (1957).
[f]Cambie et al. (1961).
[g]Martin and Martin (1982).
[h]Uprichard et al. (1975).
[i]Bate-Smith (1962).
[j]Jay (1968).
[k]McClure (1970).
[l]Santamour (1966).
[m]Berenbaum (1983).
[n]Stafford (1985).
[o]Binns et al. (1968).
[p]Jaggi and Haslam (1969).

stream ($-k$/d = 0.0040). The paucity of data for processing coefficients for leaves with intermediate and high levels of condensed tannins once again must be stressed. The last species for which there is both $-k$ values in streams and probably concentrations of condensed tannins is *Pithecellobium longifolium*. Other species in this genus contain very high levels of condensed tannins (Janzen and Waterman 1984). Fresh, undried leaves of *P. longifolium* were processed slowly ($-k$/d = 0.0048) (Stout 1980).

Cross-Latitude Leaf Transfer Experiments

In the fall of 1986, I collected autumn-abscissed leaves of white oak, *Quercus alba* (Fagaceae), from Livingston County,

TABLE 3. Tropical plant species used in Fig. 2A, with $-k/d$ values, condensed tannin levels, and references for condensed tannins (CT).

Species	Family	$-k/d$	CT	References
1. *Anaxagorea costaricensis*	Annonaceae	.0217	Low	[a]
2. *Araucaria cunninghamii*	Araucariaceae	.0198	Low	[b]
3. *Cordia borinquensis**	Boraginaceae	.0077	Low	[c]
4. *Casuarina cunninghamiana*	Casurinaceae	.0198	Low	[d, e]
5. *Buchenavia capitata*	Combretaceae	.0136	Low	[f]
6. *Nasturtium officinale**	Cruciferae	.1150	Low	[f]
7. *Sloanea berteriana**	Elaeocarpaceae	.0139	Low	[a]
8. *Aleurites montana**	Euphorbiaceae	.0173	Low	[g, g]
9. *Pithecellobium longifolium**	Fabaceae	.0048	High	[a]
10. *Myriophyllum proprinquum*	Haloragaceae	.1133	Low	[g]
11. *Liquidambar formosana*	Hamamelidaceae	.0258	Low	[d, h]
12. *Ficus glabrata**	Moraceae	.1244	Low	[d, f]
13. *Eucalyptus blakelyi*	Myrtaceae	.0172	Low	[i, j]
14. *Ardisia* sp.*	Myrsinaceae	.0040	Intermediate	[a]
15. *Manilkara bidentata*	Sapotaceae	.0111	Low	[a]
16. *Trema micrantha*	Ulmaceae	.1200	Low	[c]

*Related species analyzed for condensed tannins.
[a]Janzen and Waterman (1984).
[b]Bate-Smith (1954).
[c]Coley (1983).
[d]Bate-Smith and Metcalfe (1957).
[e]Saleh and El-Lakany (1979).
[f]Bate-Smith (1962).
[g]Cambie et al. (1961).
[h]Hatano et al. (1986).
[i]Macauley and Fox (1980).
[j]Hergert (1962).

TABLE 4. Tropical species rich in condensed tannins (CT).

Species	Family	CT (dry wt.)	Place	References
Cassia grandis	Caesalpiniaceae	43.82	Costa Rica	[a]
Hymenea courbaril		23.11		[a]
Bursera simaruba	Burseraceae	13.63		[a]
B. tomentosa		19.63		[a]
Curatella americana	Dilliniaceae	13.94		[a]
Zuelania guidonia	Flacortiaceae	16.07		[a]
Byrsonima crassifolia	Malphigiaceae	13.58		[a]
Pithecellobium oblongum	Mimosaceae	10.28		[a]
P. saman		16.05		[a]
Calycophyllum candidissimum	Rubiaceae	13.96		[a]
Luehea speciosa	Tiliaceae	17.06		[a]
Anthonotha macrophylla	Caesalpiniaceae	16.19	Camaroon	[b]
Berlinia bracteosa		11.40		[b]
Protomegabaria stapfiana	Euphorbiaceae	25.88		[b]
Garcinia ovalifolia	Guttiferae	14.44		[b]
Mammea africana		26.40		[b]
Hippocratea sp.	Hippocrateaceae	33.14		[b]
Barteria fistulosa	Passifloraceae	10.28		[b]
Cassipouria rewenzoriensis	Rhizophoroaceae	16.24	Uganda	[b]
Mimusops bagshawei	Sapotaceae	16.15		[b]

[a]Janzen and Waterman (1984).
[b]Gartlan et al. (1980).

Michigan, and tag alder, *Alnus rugosa* (Betulaceae) from Dickinson County, Michigan; leaves of oak are rated high in tannins and of alders as intermediate (see Table 2). Chamier and Dixon (1982) found that colonization rates of aquatic hyphomycetes and pectolytic bacteria were lower on oak (*Quercus robus*) and on alder (*Alnus glutinosa*), which is compatible with research showing that condensed tannins inhibit fungal colonization (Harrison 1971). In January 1987, the weighed leaf packs were taken from Michigan to Costa Rica, placed in mesh bags with 2-cm openings, and tied onto submerged wood snags in a wet tropical forest stream, the El Sura (site description in Stout 1980). At the same time, two tropical species were placed in that stream. Leaves from *Pithecellobium longifolium* (rich in condensed tannins) and *Trema micrantha* (low in condensed tannins) were oven-dried at 40°C for 48 h before weighing to approximate the physiological state of the autumn-abscissed

TABLE 5. Processing rates for leaves in native and exotic streams, leaf transfer experiment.

Species, Processing coefficient	Costa Rica	Michigan	References for Michigan
Quercus alba			
$-k$/d	.0539	.0036	[a]
		.0054	[b]
$-k$/degree day	.0039	.0014	[a]*
Alnus rugosa			
$-k$/d	.1239	.0053	[c]
		.00048 – .0086	
$-k$/degree day	.0053	.0055	
Pithcellobium longifolium			
$-k$/d	.0085	.0002	
$-k$/degree day	.00036	.000038	
Trema micrantha			
$-k$/d	.1352	.0306	
$-k$/degree day	.0057	.0036	

*Daily maximum–minimum temperatures from Dan Lawson, Michigan State University, East Lansing, USA.
[a]Suberkropp et al. (1976).
[b]Peterson and Cummins (1974).
[c]Stout et al. (1985).

mid-latitude leaves. Five replicates for each species were collected after 7, 16, 21, and 54 d; dry mass values and the percentage mass loss were determined. On August 26, 1987, oven-dried leaves of the tropical species were placed in the Ford River, Dickinson County, Michigan, along with autumn-abscissed leaves of the native *Alnus rugosa*. All leaves were tied onto bricks and five replicates for each species were collected after 7, 14, 21, 28, 52, 78, and 107 d. (Leaf processing data for the second mid-latitude species, *Quercus alba*, in its native habitat come from Augusta Creek, Michigan (after Suberkropp et al. 1976 and Petersen and Cummins 1974)).

Leaves of all except one species (*Alnus rugosa*) were processed faster in the Costa Rican stream than in the Michigan streams as determined by either $-k$/D or $-k$/degree day values (see Table 5). Based on $-k$/d values, three findings were made. First, leaves of *Quercus alba* were processed one hundred times faster in the Costa Rican stream than in Augusta Creek, Michigan. In fact, the processing rate in Costa Rica approached the leaching rate of this species. (This rate equals dry mass loss during the first 24 to 48 h, usually a 20 to 40% loss of original mass (Cummins et al. 1989)). Second, leaves of the mid-latitude species lower in tannins, *Alnus rugosa*, were processed in the El Sura at one of the fastest rates recorded for deciduous leaves (see Webster and Benfield 1986). Third, dried leaves of both tropical species were processed much more slowly in the Ford River than in the Sura. *Trema micrantha* (tropical) and *Alnus rugosa* (mid-latitude), which have the highest processing coefficients, are low and intermediate, respectively, in tannin concentrations (see Table 3).

As mean stream temperatures of the Sura differ widely from the Ford River and Augusta Creek, $-k$/degree day values were computed to normalize temperature differences (see Table 5). Rate categories defined by Petersen and Cummins (1974) are for $-k$/d and not for $-k$/degree day values. For heuristic purposes, those categories are used below for replacement of processing rates determined by $-k$/degree days.

According to degree days, *Quercus alba* was processed "slowly" in Costa Rica and Michigan. *Pithecellobium longifolium* was processed "very slowly" at both sites. Both species are high in tannins. *Alnus rugosa* was processed at "intermediate" rates in Michigan and Costa Rica. *Trema micrantha* was processed "slowly" in Michigan and at an "intermediate" rate in Costa Rica.

After differences in water temperature were normalized by using degree days, only leaves of *P. longifolium* were processed substantially slower in Michigan than in Costa Rica. Although only two species of the genus have been analyzed for condensed tannins, they range from containing more than 10% to more than 16% by dry mass of condensed tannins. It appears probable that *P. longifolium* also is very rich in condensed tannins. The second species used in the present study that is tannin-rich is *Q. alba*. Its reported tannin content is relative to other species in the genus; even so, its tannin content is much lower than 10% (Martin and Martin 1982). Its degree day processing rates, although slower in Michigan than in Costa Rica, were not an order of magnitude different between the two sites.

It is intriguing to speculate that the defensive compound accounts for the slower processing rates in Michigan. Very high concentrations in leaves of some tropical species may significantly slow processing rates, especially in exotic areas such as Michigan where the biota have not experienced the quantity or array of tropical defensive compounds. In future studies, described below, the exchange among tropical, mid-latitude and boreal regions of leaves rich and poor in condensed tannins may address these speculative statements.

At a workshop entitled Factors Controlling Community Structure and Function in Tropical versus Temperate Streams (Flathead Lake, Montana, April 1987), six scientists decided to exchange leaves of two plant species from each of their research sites; one species theorized as being high and the other known to be low in condensed tannins. The experimental design is specifically intended to compare processing rates of these leaves in native and "exotic" streams. Six sites located in Costa Rica, Puerto Rico, North Carolina, New York, Michigan, and Alaska, will be used. Data from these experiments will be the first direct tests of the hypothesis.

Terrestrial decomposition studies show that, overall, mid-latitude leaf processing rates are slower than tropical rates, although there is "considerable" overlap between litter decomposition rates for the slowest tropical species and the fastest temperate species" (Anderson and Swift 1983). Would the results be the same if variances in soil temperature were normalized? Variances not attributable to temperature may include the effects of phytochemical and physical protective mechanisms in terms of herbivory and microbial decay. Calculation of processing rates using $-k$/degree days may reveal those effects. Such investigations will require collection of maximum–minimum daily (or possibly weekly) temperatures.

Conclusions

Studies showing that condensed tannins affect decomposition and microbial activity (Benoit et al. 1968; Harrison 1971; Grant 1976) are sufficiently compelling to promote stream ecologists to look at these concentrations in plant tissues. Understanding action of condensed tannins in aquatic ecosystems, especially at the microorganismic level, could provide a fresh perspective on plant–animal interactions in aquatic food chains. If cross–latitudinal comparisons are done, daily monitoring, if possible, of maximum–minimum water temperature will be necessary in order to normalize latitudinal differences. Weekly monitoring would provide useful but less precise information.

The underlying functional effect of condensed tannins on aquatic fungal and bacterial populations probably will not be understood without condensed tannin bioassays different from those being developed to study the effects of tannins on vertebrates and insects (Martin and Martin 1982, 1983; Mole and Waterman 1987a, 1987b).

Methods for determining condensed tannin concentrations are varied; for example, Binns et al. (1968); Bate-Smith (1977); and Stafford (1985). Laboratories using the same methods of analysis may differ in technique, making utilization of data from various sources difficult. Until methods are standardized, the best approach would be to have analyses done in a single laboratory.

Studies on the dynamics of leaf inputs in mid-latitude streams lack specific data on secondary compounds. Although tropical ecologists working on terrestrial sites have such data for many plant species, little information on processing rates of leaves in tropical streams is available. Baseline data on the phytochemistry of riparian vegetation and on leaf processing in tropical streams should be obtained before tropical watersheds are severely disturbed by herding, timbering, and agricultural practices (Lugo and Brown 1982; Myers 1980; Buschbacher 1986; Janzen 1986; Tangley 1986a, 1986b). Accumulation of these sorts of data may add robustness and precision to the River Continuum Theory (Vannotte et al. 1980), as the chemistry of allochthonous inputs may control or influence aquatic communities dependent on them more than does stream order designation. This could be especially relevant for the biomes of watersheds where soils are low in available nutrients. Research on plant–animal interactions in rheophilic systems also could be enhanced by studying phytochemical ecology in streams.

Acknowledgments

This work was supported by a grant from the U.S. Department of the Navy, Contract #N000039-81-C-0357. Help from students in the 1987 Fundamentals of Tropical Biology Course, sponsored by the Organization for Tropical Studies is warmly appreciated. Thanks for comments on the manuscript go to Bill Cooper, Don Hall, H. B. N. Hynes (a reviewer), Jack Stanford, John Vandermeer, and Peter Waterman. Discussions at the April 1987 Flathead Lake Workshop spurred the writing of this manuscript.

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Geographic Variation in the Occurrence of Red- and White-Fleshed Chinook Salmon (*Oncorhynchus tshawytscha*) in Western North America

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Hard, J. J., A. C. Wertheimer, and W. F. Johnson. 1989. Geographic variation in the occurrence of red- and white-fleshed chinook salmon (*Oncorhynchus tshawytscha*) in western North America. Can. J. Fish. Aquat. Sci. 46: 1107–1113.

Flesh color, as reported by fishery processors, was surveyed in coded-wire tagged chinook salmon (*Oncorhynchus tshawytscha*) sampled from the 1983–85 Southeast Alaska commercial troll fisheries. Flesh color data were collected from 11 373 fish originating from 95 locations in western North America between southcentral Alaska and central California. White-fleshed chinook salmon were most common in stocks from coastal British Columbia, Southeast Alaska, and the tributaries of the Fraser River, British Columbia. Heterogeneity between neighboring locations was also greatest in this region, the most recently glaciated area of western North America. Differences in flesh color proportions are consistent with the hypothesis that chinook from the Pacific refuge colonized rivers in British Columbia and Southeast Alaska after the recession of the last Pleistocene glaciation. Genetic drift of populations founded by small numbers of parents is one explanation of the pattern of occurrence of white-fleshed chinook salmon. An alternate explanation is that geographic variation in the occurrence of red- and white-fleshed chinook salmon reflects evolutionary adaptations of these fish during early life history to local freshwater environments.

On a étudié la couleur de saumons chinook (*Oncorhynchus tshawytsha*) capturés à la traine de 1983 à 1985 dans les pêcheries d'Alaska. Les employés des usines ont indiqué la couleur de 11 373 poissons étiquetés provenant de 95 stations de l'Alaska au centre de la Californie. Les saumons à chair blanche étaient surtout fréquents dans les stocks provenant de la côte de Colombie-Britannique, du sud-est de l'Alaska et des affluents du Fraser (C.-B.). C'est dans cette région, la plus récemment déglaciée de l'ouest de l'Amérique du Nord, que les stations étaient le plus hétérogènes. Les différences entre les proportions de couleurs concordent avec l'hypothèse voulant que le chinook provenant du refuge du Pacifique ait colonisé les rivières de la Colombie et du sud-est de l'Alaska après le retrait des derniers glaciers du Pléistocène. La différentiation génétique des populations issues d'un petit nombre de parents est une des explications des modes de fréquences du chinook à chair blanche. Autre explication possible : la variation géographique de la fréquence des chinook à chair rose et à chair blanche refléterait une adaptation précoce à leurs milieux d'eau douce particuliers.

Received August 10, 1988
Accepted March 9, 1989
(J9842)

Reçu le 10 août 1988
Accepté le 9 mars 1989

Some characteristics of a species vary among its spatially segregated populations. While dispersal and genetic drift contribute to differentiation of populations, much of this variation is thought to represent adaptations to local environments (Mayr 1963; Marcus and Vandermeer 1966; Gould and Johnston 1972). Stocks of chinook salmon (*Oncorhynchus tshawytscha*) show considerable variation in several life history traits, including fecundity (McGregor 1923; Rounsefell 1957; Healey and Heard 1984), length of residence in freshwater (Healey 1983), and age at maturity (Ellis and Noble 1961; Hard et al. 1985).

Chinook salmon stocks also vary in flesh color (Hard 1986; Withler 1986). As in coho salmon (*O. kisutch*) (Iwamoto and Hershberger 1984) and rainbow trout (*O. mykiss*) (Torrissen and Naevdal 1984; Torrissen 1985), flesh coloration of chinook is heritable but is dependent upon dietary intake of carotenoid pigments for expression (Withler 1986; McCallum et al. 1987). Chinook salmon are unique among Pacific salmon (*Oncorhynchus* spp.) because immature fish feeding at sea develop one of two distinct flesh colors; the two forms are known as red- and white-fleshed (Milne 1964; Godfrey 1968, 1975). Genetic control of these color types is remarkably strong; heritability of this trait, when treated as a threshold character, was 0.8 for Quesnel River chinook salmon, based on the mean of dam and sire component estimates reported by Withler (1986). Flesh coloration

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in chinook salmon is treated as a dichotomous trait by the fishing and processing industries; fish with detectable flesh color, ranging from pale pink to deep red, are typically considered red-fleshed. Red-fleshed chinook are usually more valuable; for example, in the Southeast Alaska troll fishery in 1984–85, the ratio of values of red- to white-fleshed chinook to the commercial troller averaged 1.5 to 1 (Statistics and Market News Office, National Marine Fisheries Service, Seattle, WA, unpubl. data).

In this paper we describe the geographic distributions of red- and white-fleshed chinook salmon throughout much of the species' natural range. We attempt to explain these patterns in light of hypotheses on the dispersal of chinook salmon since the last Pleistocene glaciation and on the possible adaptive significance of egg pigmentation, a trait correlated with flesh coloration (Hard 1986).

Methods

Fish Sampling

The Alaska Department of Fish and Game (ADFG) annually samples the commercial troll fishery in Southeast Alaska for coded-wire tagged (Jefferts et al. 1963) chinook salmon to determine the contributions of various releases to this fishery. Chinook salmon are sampled when the fish are sold to processors. The processors grade these fish as red- or white-fleshed after visual inspection of the flesh. While grading is subjective and may vary among processors, errors in the process are difficult to quantify (Godfrey 1975) and are not considered in this report. Since 1983, ADFG personnel have included flesh color information in their fishery samples. Because the origin of a fish can be established from the coded data on its wire tag, the collection of flesh color information from individual tagged fish allows stock differences in flesh color proportions to be estimated.

In order to measure geographic variation in the occurrence of red- and white-fleshed chinook salmon, we surveyed recoveries of 9865 coded-wire tagged chinook salmon in the 1983–85 Southeast Alaska commercial and sport fisheries that included those released from 95 locations between southcentral Alaska and central California. We also used flesh color data from the river mouth sport fishery on the Chilkat River, Alaska from 1982, 1984, and 1985 (P. D. Kissner, ADFG, Division of Sport Fisheries, Juneau, Alaska, unpubl. data). Although flesh color of chinook salmon loses its intensity during maturation, it can be accurately predicted from skin and egg colors (Hard 1986). Such criteria were used to assess flesh color of 1508 spawning chinook salmon on the Taku River in 1985 (P. D. Kissner, ADFG, Division of Sport Fisheries, Juneau, Alaska, unpubl. data), and these data were included in the analysis. Information on chinook salmon from rivers outside the geographic range contributing tagged fish to the Southeast Alaska fisheries was provided by biologists and processors familiar with the fisheries of these rivers.

Analysis

Recoveries of chinook from several releases were small. Under the assumption that the assignment of flesh color is binomially distributed, a random sample of 60 fish is required to detect at least one white-fleshed fish in the sample ($P<0.05$), if 5% of the population are actually white-fleshed. Assuming that genetic differences among stocks are temporally stable (Gharrett et al. 1987), we combined annual recoveries of chinook from each release location to increase the sizes of small samples. Where sufficient data ($n\geqq60$) were available in each recovery year, we tested for differences in flesh color proportions between years (see below). For all recovery years, the proportions of red- and white-fleshed fish originating from neighboring sites were tested for homogeneity with the G-test of independence (Sokal and Rohlf 1981); these recoveries were combined if they were not heterogeneous ($P>0.05$). This procedure defined a locality that was the geographic center of the pooled sites (Gabriel and Sokal 1969). Any two given localities A and B were considered contiguous if a circle of diameter AB with A and B on its circumference included no other localities (Gabriel and Sokal 1969).

Regional heterogeneity within the entire data set was tested with a simultaneous test procedure (STP) for categorical data (Gabriel 1966; Gabriel and Sokal 1969). The STP employed the likelihood-ratio test statistic G, adjusted with Williams' correction (Sokal and Rohlf 1981), whose critical value for all tests was determined by the test of homogeneity among localities for the entire data set. If the null hypothesis of homogeneity within this set was rejected, the critical value of G obtained from this test was used as the rejection criterion for all subsequent hypotheses involving subsets of contiguous localities. The results of the STP were then used to construct "maximal acceptable connected subsets," the largest possible homogeneous regions of connected localities such that no region can be a subset of another (Gabriel and Sokal 1969). Three major geographic areas were first tested for homogeneity: Southeast Alaska, British Columbia, and the northwestern contiguous United States. If hetereogeneity was detected within these areas, they were partitioned into homogeneous subsets. Contiguous localities from adjacent areas were then added to these subsets until heterogeneity was again detected. With the STP, the probability of falsely accepting the null hypothesis of homogeneity when the localities are in fact heterogeneous (type II error) is increased because several hypotheses are tested simultaneously, and thus some real differences may not be detected. However, the nature of the STP ensures that the probability of falsely rejecting homogeneity (type I error) does not exceed the desired level (in this analysis, we use $P<0.05$).

There were sufficient recoveries of fish (i.e. $n\geqq60$) from nine origins to test if the proportion of white-fleshed chinook salmon varied among recovery years. For four of the sites, the recoveries of white-fleshed fish were sufficient to use the G-test to examine for between-year differences. For the other five sites, where white-fleshed fish were rare, the expected frequencies of white-fleshed fish were too low to permit use of the G-test. In these cases, the probability of occurrence for each year (including the probability of even more extreme results) was computed, assuming the true probability was equal to the mean proportion of white-fleshed fish observed for that site over all years. If the probability of obtaining the observed or a smaller number of white-fleshed fish for any year was less than 0.05, then the null hypothesis of no difference between years was rejected. Differences between the brood years that contributed to these recoveries were then tested in a similar manner.

Results

Overall Heterogeneity in Flesh Color Proportions

Twenty-one localities were defined within the geographic range of stocks that contributed to the Southeast Alaska chinook

TABLE 1. Proportions of white-fleshed chinook salmon recovered in Southeast Alaska commercial and sport fisheries from 21 localities in western North America. The localities are arranged from north (1) to south (21); their geographic locations are shown in Fig. 1. Localities with the same letter preceeding them belong to the same maximal acceptable subset. White-fleshed scores are relative (+, −) to the overall mean.

Locality			White-fleshed chinook salmon recovered			
No.		Name	No.	%	Score	Total
1	A	Chilkat River, AK	174	37.1	+	468
2	B	Taku River, AK	183	12.0	+	1528[a]
3	C	Stikine River, AK	23	2.6	−	893
4	D	Unuk River, AK	505	16.3	+	3094
5	D	Chickamin River, AK	29	17.4	+	167
6	E	Upper Skeena River, B.C.	0	0.0	−	61
7	F	Lower Skeena River, B.C.	134	41.2	+	325
8	E	Bella Coola River, B.C.	6	8.1	−	74
9	E	Upper Fraser River, B.C.	1	4.2	−	24
10	E,H	Southern Coastal B.C.	1	4.2	−	24
11	G	Lower Fraser River, B.C.	35	53.8	+	65
12	E,H	East Vancouver Island, B.C.	20	2.1	−	936
13	E	West Vancouver Island, B.C.	7	0.5	−	1509
14	H	Northwestern Washington	8	3.6	−	220
15	H	Priest Rapids, Columbia River	4	1.5	−	262
16	H	Snake River, ID	2	4.8	−	42
17	H	Lower Columbia River	2	0.8	−	245
18	H	Mid-Columbia River	3	0.6	−	494
19	H	Northern Coastal Oregon	0	0.0	−	196
20	H	Willamette River, OR	3	0.5	−	613
21	H	Southern Coastal OR	2	1.5	−	133
		Total	1142	$\bar{X}=10.1$		11 373[b]

[a]Numbers include 1508 spawning chinook salmon (P. D. Kissner, ADFG, Juneau, AK, unpubl. data) as well as 20 coded-wire tagged fish recovered in sport and commercial fisheries.

[b]An additional 25 chinook salmon were recovered in the commercial troll fishery but were not included in the analysis because of their poor representation and isolated sources: three fish from the Kenai Peninsula, AK; 20 fish from Massett Inlet, Queen Charlotte Islands, B.C.; and two fish from the Feather River, CA. All fish but one (Massett Inlet) were red-fleshed.

salmon fishery (Table 1). The proportion of white-fleshed fish in the recoveries varied from 0% in the upper Skeena River to 53.8% in the lower Fraser River. The overall mean of the proportion of white-fleshed chinook salmon in the 21 localities was 10.1%. The proportion of white-fleshed chinook salmon exceeded the mean in six localities: four short coastal rivers in Southeast Alaska (Chilkat, Taku, Unuk, and Chickamin) and the lower sections of two rivers in British Columbia (Skeena, Fraser). Based on the STP procedure, the 21 localities showed significant heterogeneity ($P<0.005$) in their proportions of red- and white-fleshed chinook salmon. White-fleshed chinook salmon are evidently rare in rivers outside the geographic range of stocks surveyed in the fishery, i.e. south of Oregon and north of the Chilkat River in Southeast Alaska (Table 2).

Regional Comparisons

Heterogeneity among contiguous localities was greatest in Southeast Alaska where the five identified localities (1–5, Table 1) could be partitioned into four maximal acceptable subsets, three containing one independent locality each and one containing two localities. In British Columbia, six of eight localities identified (6–13, Table 1) could be grouped as a subset; the other two localities, the lower Skeena and lower Fraser Rivers, were independent of their contiguous localities. All eight localities identified in the contiguous United States (14–21, Table 1) could be grouped as a single subset. Two contiguous British Columbia localities, southern coastal British Columbia and East Vancouver Island, could also be added to this subset without detecting significant heterogeneity among localities (Table 1).

The occurrence of white-fleshed chinook salmon in North America was common only in areas most recently covered by ice during the Pleistocene epoch (Fig. 1). Flesh-color heterogeneity for the localities for which it was quantified was greatest in recently glaciated areas.

Comparisons among Years

Of the nine locations for which sufficient data existed to examine differences among recovery years in the frequencies of white-fleshed chinook salmon, three showed significant ($P<0.05$) variation among years (Table 3). For two of these locations, there was no difference ($P>0.10$) in the frequencies of white-fleshed chinook salmon when the recoveries are examined by brood year (Table 4). However, differences observed in fish from the Unuk River stock of chinook salmon released at Little Port Walter were attributable to strong variation ($P<0.01$) in the frequencies of white-fleshed fish among brood years. The frequency of white-fleshed chinook salmon from Little Port Walter was dramatically lower in 1983 and 1984 than those from the same donor stock released at Whitman Lake and Deer Mountain hatcheries (Table 3).

TABLE 2. River systems in western North America where white-fleshed chinook salmon are rarely observed. These systems contributed no tagged fish to the survey of the Southeast Alaska fisheries; all sources are personal communications with biologists and processors having extensive experience with the fisheries and fish populations of these rivers. Letters in parentheses refer to sites on Fig. 1.

System	Sources of information	
	Name	Agency[a]
Western Alaska		
Yukon (A), Nushagak, Togiak	L. Buklis	ADFG-CF, Anchorage, AK
	M. Nelson	ADFG-CF, Dillingham, AK
Southcentral Alaska		
[b]Kenai (B),	K. Delaney	ADFG-SF, Anchorage, AK
[b]Susitna	P. Kissner	ADFG-SF, Juneau, AK
	D. Nelson	ADFG-SF, Soldotna, AK
Copper (C)	M. McCurdy	ADFG-CF, Cordova, AK
	K. Rochhildt	NPP, Cordova, AK
Southeast Alaska		
[b]Alsek (D)	P. Kissner	ADFG-SF, Juneau, AK
	B. McGuire	SSF, Sitka, AK
California		
Klamath (E)	L. Boydstun	CDFG, Rancho Cordova, CA
Sacramento (F)	J. Lesh	CDFG, Eureka, CA
	L. Amundsen	PSGF, Santa Rosa, CA

[a]Listing of agency acronyms:
ADFG-CF: Alaska Department of Fish and Game, Division of Commercial Fisheries,
ADFG-SF: Alaska Department of Fish and Game, Division of Sport Fish,
NPP: North Pacific Processors,
SSF: Sitka Sound Seafoods,
CDFG: California Department of Fish and Game,
PSGF: Point St. George Fisheries.

[b]Marsh and Cobb (1908) noted that chinook salmon returning to Cook Inlet rivers are "red-meated," and that "white-meated" chinook salmon occur in the Alsek River.

Discussion

The interpretation of flesh-color data from coded-wire tagged chinook salmon is influenced by the heavy reliance on hatchery stocks to represent geographic locales. A much higher proportion of the hatchery production of chinook salmon is tagged relative to production of wild fish. We assume that hatchery chinook salmon are, in terms of flesh color, representative of chinook salmon populations in the geographic locales where they were released. While there is no indication of selective breeding for flesh color except for research (Hard 1986; Withler 1986; McCallum et al. 1987), small founder populations, transplants between hatcheries, and pooling of tag releases within a region to increase sample sizes, could obscure real differences in flesh color among stocks and regions. However, our use of these data is supported by the fact that the pattern of occurrence of white-fleshed chinook salmon that we observed in the tag–recovery data is consistent with published accounts of their geographic range: white-fleshed chinook salmon have been reported from various river systems in Southeast Alaska and British Columbia, and are rarely noted in stocks north and south of these regions (Marsh and Cobb 1908; Milne 1964; Godfrey 1975; Fraser et al. 1982).

Chinook salmon probably survived glaciation in both northern (Bering) and southern (Pacific) refugia (McPhail and Lindsey 1970). Anadromy was well developed in *Onchorhynchus* spp. by the Pleistocene epoch (Tchernavin 1939; Neave 1958; Cavender and Miller 1972); colonization of British Columbia and Southeast Alaska from the Pacific refugium could therefore have occurred rapidly via sea routes (McPhail and Lindsey 1986a, 1986b). White-fleshed chinook salmon do occur in portions of the geographic area of the Pacific refugium, suggesting that this area served as a genetic reservoir from which chinook salmon invaded coastal British Columbia and Southeast Alaska. This does not exclude colonization of portions of the glaciated area by chinook salmon originating in the Bering refugium. Based on electrophoretic evidence, Gharrett et al. (1987) found that chinook salmon in Southeast Alaska were intermediate in genetic difference between those from western Alaska and the Pacific Northwest, and were more heterogeneous than were western Alaska stocks. They concluded that dispersal of chinook salmon to Southeast Alaska since the last glaciation probably occurred from both the Pacific and Bering refugia.

Inference about the patterns of heterogeneity in flesh-color frequencies requires the assumption that the observed differences reflect genetic differences among stocks which are temporally stable. We found only one case where there was significant variation in flesh-color frequencies among both recovery and brood years: chinook salmon from the Unuk River released at Little Port Walter. The same stock of chinook salmon released at other sites did not show such variability in flesh-color frequencies, suggesting that the differences were due to small founder populations that were not representative of the donor stock.

Genetic drift of small founder populations following postglacial dispersion is one explanation for the extreme heterogeneity of flesh color among stocks of chinook salmon. The variability of flesh color observed for the Unuk River chinook salmon transplanted to Little Port Walter is indicative of how small subsamples of parents from a population can result in significantly different genetic frequencies; over several thousand years, isolation and genetic drift in small populations could further emphasize divergence (Dillon 1984). If some river systems in Southeast Alaska were colonized by chinook salmon from the Bering refugium as well as the Pacific refugium, the differential gene flow could contribute to the heterogeneity in the glaciated areas. For example, the Stikine River had a low frequency of white-fleshed fish relative to other river systems in Southeast Alaska (Table 1), and there is zoogeographical evidence of colonization of the Stikine River via interior routes from the Bering refugium (McPhail and Lindsey 1986a).

An alternate explanation of the distribution of white-fleshed chinook salmon is that natural selection restricts these fish to a limited geographic range and to certain habitats within that range. Chinook salmon are ecologically rather specialized; their freshwater habitat is typically limited to large drainages (Ricker 1972). Chinook salmon stocks with high proportions of white-fleshed fish are further restricted to coastal sections of large rivers in British Columbia and short coastal rivers in Southeast Alaska. Within the Fraser River system, white-fleshed chinook salmon spawn mainly in the lower river tributaries, while red-fleshed chinook salmon are predominate in the upper river (Milne 1964; Godfrey 1975). Coastal watersheds usually have cooler summer temperatures and higher fall and winter precipitation than inland systems (Northcote and Larkin 1956; Anonymous 1971; Stockner and Shortreed 1983). If genetic drift of

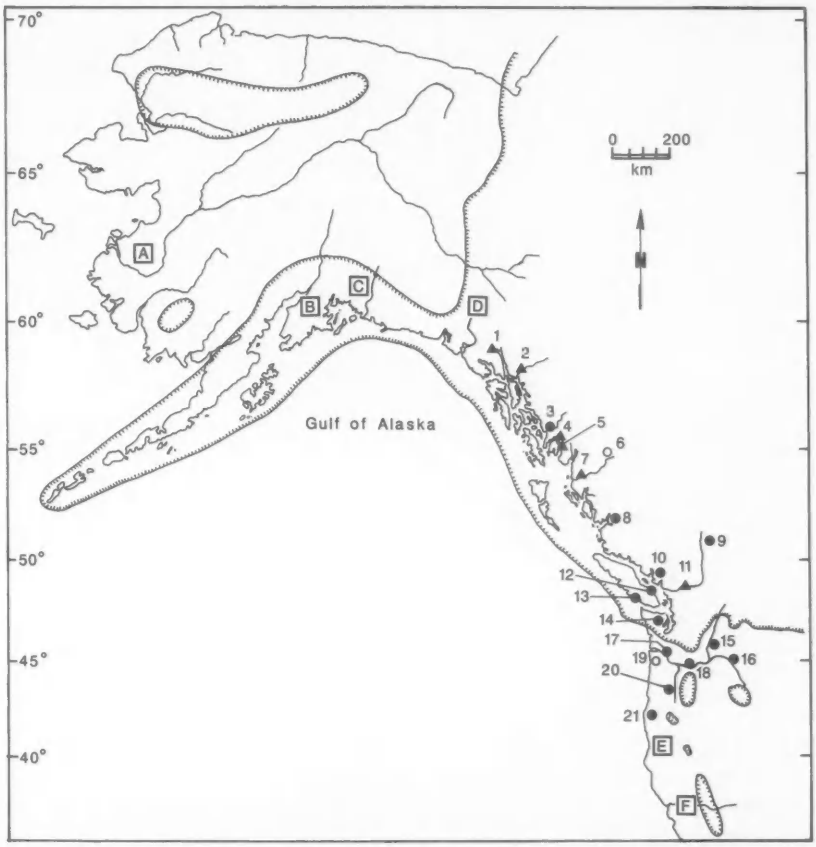

FIG. 1. Occurrence of white-fleshed chinook salmon in western North America in relation to former glaciation patterns. The areas enclosed by cross-hatched lines represent the extent of the Wisconsin glaciation during the Pleistocene epoch (after McPhail and Lindsey 1970). Localities denoted by ▲'s had observed proportions of white-fleshed chinook salmon higher than the overall mean; those denoted by ● had lower than average proportions; and those denoted by ○ had no white-fleshed chinook salmon observed (see Table 1 for key to locality numbers). The letters A–F denote rivers that contributed no tagged fish to the fisheries sampled for flesh color in this study, but in which white-fleshed chinook salmon are reportedly rare (see Table 2 for key).

TABLE 3. Proportions of white-fleshed chinook salmon recovered by year, originating from nine locations in western North America. For a given location, years with percentages that share the same letter in superscript are not significantly different ($P>0.05$) in these proportions.

	1983		1984		1985	
Location (stock)	Total recovered	% white	Total recovered	% white	Total recovered	% white
Chilkat River, AK	140	38.6[a]	151	41.1[a]	177	32.3[a]
Little Port Walter, AK (Unuk River)	327	4.9[a]	345	8.4[a]	262	19.8[b]
Deer Mountain, AK (Unuk River)	497	14.9[a]	230	16.1[a]	185	20.0[a]
Whitman Lake, AK (Unuk River)			297	15.1[a]	912	23.1[b]
Quinsam River, B.C.	111	2.7[a]	132	4.5[a]	464	1.7[a]
Robertson Creek, B.C.	626	0.3[a]	376	0.3[a]	90	3.3[b]
Priest Rapids, WA			102	0.0[a]	106	3.8[a]
Bonneville, OR	157	1.9[a]	166	0.0[a]	151	0.0[a]
McKenzie River, OR	93	0.0[a]			119	1.7[a]

TABLE 4. Proportions of white-fleshed chinook salmon recovered by brood year for three release sites. These sites were identified as having significant heterogeneity among recovery years (Table 3). For a particular site, years with the same letter in superscript are not significantly different ($P>0.05$) in the frequencies observed.

	1978		1979		1980		1981	
Location (stock)	*N*	%	*N*	%	*N*	%	*N*	%
Little Port Walter, AK (Unuk River)	423	6.4[a]	232	7.3[a]	80	8.8[a]	199	23.1[b]
Whitman Lake, AK	—	—	—	—	549	19.6[a]	396	23.3[a]
Robertson Creek, B.C.	140	0.0[a]	494	0.2[a]	312	1.3[a]	68	1.5[a]

small founder populations was the sole source of the heterogeneity in the occurrence of white-fleshed chinook salmon, then their distribution should not be associated with certain types of watersheds but randomly distributed throughout the region in which they exist.

A possible mechanism for natural selection is a relationship between egg pigmentation and embryonic survival. Egg pigmentation in salmonids may ameliorate mortality of embryos developing under harsh environmental conditions (Mikulin and Soin 1975; Tacon 1981; Craik 1985). Carotenoids aid in respiration of eggs of several fishes in environments where available oxygen is at a low concentration (Hubbs and Stavenhagen 1958; Balon 1977; Craik 1985). Soin (1956) speculated that variation in egg coloration among three species of *Oncorhynchus* in Asia results from adaptation to specific spawning and incubation environments. The darker pigmentation of eggs of red-fleshed chinook salmon (Hard 1986) may provide an advantage to the embryos under some environmental conditions. For example, high summer temperatures or low winter streamflows could reduce available oxygen in the incubation environment.

If there is a selective advantage to the carotenoid content of red-fleshed chinook salmon, the persistence of white-fleshed fish suggests that red flesh incurs a cost; this cost may be reduced size at maturity. In British Columbia gill-net catches of adult chinook salmon, white-fleshed chinook salmon are consistently larger than red-fleshed fish (Milne 1964; Godfrey 1975; Fraser et al. 1982). Greater size in chinook salmon has value in both increased survivorship and reproductive value (Healey and Heard 1984). If this is indeed the trade off, then selection for red pigmentation must be more intense than selection for increased size in most habitats, particularly in the periphery of the species range. If these traits are negatively correlated, selection on each would act to maintain genetic variance in both traits. Further research into the relationship between flesh color and life history characteristics such as survivorship, fecundity, and breeding size, and the relationship between embryonic carotenoid content and survival during early development, is needed to confirm whether flesh color of chinook salmon is the result of an adaptive response to natural selection, or simply an artifact of postglacial dispersal and genetic drift.

Acknowledgments

We thank Paul Kissner for his interest and insights, as well as the data he contributed to this study. We also thank Drs. Tony Gharrett, William Bradshaw, Christina Holzapfel, and two anonymous referees for their thoughtful reviews of this manuscript.

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Development and Transfer of Plasmid-Mediated Antimicrobial Resistance in *Edwardsiella ictaluri*

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Edwardsiella ictaluri were found to develop and transfer plasmid-mediated antimicrobial resistance. All isolates tested were found to receive resistance(*R*)-plasmids at frequencies ranging from 10^{-3} to 10^{-6}. The antimicrobials to which *E. ictaluri* became resistant included tetracycline and Romet, the only two antibiotics cleared for use in channel catfish (*Ictalurus punctatus*) culture. The frequency of plasmid transfer appeared to be temperature related, with higher frequencies at 30°C than at 20 or 37°C. *E. ictaluri* having received an *R*-plasmid was shown to transfer the plasmid to *Escherichia coli* and *Yersinia ruckeri* at fairly high frequencies.

On a découvert qu'*Edwardsiella ictaluri* développe et transmet un plasmide de résistance antimicrobienne. On a trouvé que tous les isolats testés recevaient des plasmides de résistance à des fréquences variant entre 10^{-3} et 10^{-6}. La résistance antimicrobienne s'applique notamment à la tétracycline et au Romet, les deux seuls antibiotiques autorisés dans l'élevage commercial de la barbue de rivière. La fréquence du transfert de plasmide semble reliée à la température; elle est plus élevée à 30 qu'à 20 ou 37 degrés C. On a trouvé que la barbue ayant reçu un R-plasmide le transférait à *Escherichia coli* et à *Yersinia ruckeri* à une fréquence passablement élevée.

Received August 12, 1988
Accepted March 9, 1989
(J9847)

Reçu le 12 août 1988
Accepté le 9 mars 1989

Edwardsiella ictaluri, the etiologic agent in enteric septicemia of catfish (ESC), is the leading cause of bacterial mortality in cultured catfish in the United States. In spite of recent advances in understanding the pathogenesis of ESC (Blazer et al. 1985; Shotts et al. 1986) and possible virulence mechanisms of *E. ictaluri* (Waltman et al. 1986), the incidence of ESC is increasing. Currently, control measures consist of a combination of management practises and antibiotic treatment. The only antibiotics currently approved for use in channel catfish (*Ictalurus punctatus*) culture are tetracycline and Romet. *Edwardsiella ictaluri* has been shown to be susceptible to both of these antibiotics (Waltman et al. 1986). The widespread use of tetracyclines in fish culture has resulted in the development of plasmid-mediated antibiotic resistance in several fish pathogens (Aoki et al. 1971; Aoki et al. 1974; Aoki et al. 1977; Shotts et al. 1976). Consequently, this study was undertaken to determine the potential development of plasmid-mediated antibiotic resistance in *E. ictaluri*.

Materials and Methods

Isolates

Five representative isolates of *E. ictaluri* were selected from our culture collection (Waltman et al. 1986) for study. *Edwardsiella ictaluri* 1 is the American Type Culture Collection (ATCC 33202) strain isolated in 1977. Isolate 19 was isolated in 1982 from a *Danio* (Waltman et al. 1985). Isolates 557, 884, and 301 were isolated in Mississippi in 1982, 1983, and 1984, respectively. *Escherichia coli* 1932 (lactose negative, nalidixic acid resistant) was used as the recipient control organism. *Escherichia coli* 1898, a multiply-resistant organism isolated from an equine urinary tract infection (Wooley et al. 1986), was used as the source of an antibiotic resistance (*R*) plasmid for these studies.

R-Plasmid Transfer

The transfer of the *R*-plasmid was accomplished using an adaptation of the method of Watanabe (1964). Briefly, donor and recipient isolates were grown in penassay broth (Difco Laboratories, Detroit, MI), pH 7.6, for 18–20 h at 30°C. Equal volumes of each culture (usually 1 mL) were mixed together and incubated for 18–20 h at 30°C. Transconjugates were selected by quantitative plating onto MacConkey (MAC) or Mueller Hinton (MH) agars containing antibiotics. For the *E. coli* 1898/*E. coli* 1932 mating, transconjugates were selected using MAC containing 30 μg/mL nalidixic acid (NA) plus one of the following: 30 μg/mL tetracycline (TET), 30 μg/mL chloramphenicol (CHLR), 30 μg/mL kanamycin (KAN), or 10 μg/mL ampicillin (AMP), and MH containing 30 μg/mL NA and 25 μg/mL Romet (ROM). The *E. coli* 1898/*E.* ictaluri transconjugates were selected using MAC plus 10 μg/mL colistin (COL) with TET, CHLR, KAN, or AMP as above, and MH plus 10 μg/mL COL and ROM. The resulting bacterial colonies were subcultured, identified by the methods described by Waltman et al. (1986), and examined for antimicrobial susceptibility. Frequency of plasmid transfer was expressed relative to the number of donor cells in the mating mixture (Watanabe 1964). Determination of possible transductional passage of

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resistance was accomplished by mixing the recipient cells with the cell-free broth culture from the donor organism and processing as above. The relationship of temperature to the frequency of *R*-plasmid transfer was determined by conducting matings at 20, 30, and 37°C.

Antimicrobial Susceptibility

Antimicrobial susceptibilities of donor, recipient, and transconjugate organisms were determined using the disk method of Bauer et al. (1966), except that the incubation temperature was 30°C. After each bacterial isolate was grown to a standard density and spread over a MH plate, the respective antimicrobial disks were added. The following antimicrobials were tested: TET (30 μg disk), CHLR (30 μg disk), streptomycin (STR, 10 μg disk), KAN (30 μg disk), trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 μg disk), ROM (25μg disk), AMP (10 μg disk), cephalothin (CEPH, 30 μg disk), NAL (30 μg disk), and COL (10 μg disk). All the antibiotic disks, except ROM, were purchased from BBL (BBL Microbiology Systems, Cockeysville, MD); ROM was obtained from Hoffman-LaRoche (Nutley, NJ). After incubation at 30°C the zones of growth were measured and the susceptibility of the organism to each antimicrobial was interpreted as recommended by BBL Sensi-Disc Antimicrobial Susceptibility Test Discs package insert, May 1986.

Agarose Gel Electrophoresis

Plasmid DNA was extracted from selected donor, recipient, and transconjugate isolates following the method of Hansen and Olsen (1978). Samples (10 μL) were electrophoresed in 0.7% agarose gels at 100 V for 90 min in a mini-sub DNA gel apparatus (Bio-Rad, Richmond, CA) with a constant power source (E-C model 452, E-C Apparatus Corp., St. Petersburg, FL.). Gels were stained with ethidium bromide, and plasmid bands were visualized on an ultraviolet transilluminator (Model TM 36, Ultraviolet Products, Inc., San Gabriel, CA). Photographs were taken with Polaroid type 35 film (Polaroid Corp., Cambridge, MA) using a No. 23A Wratten gelatin filter (Eastman Kodak Co., Rochester, NY) with a Polaroid MP4 land camera.

Results

Escherichia coli 1898 was resistant to all antimicrobials tested except NA and COL. *Escherichia coli* 1932 was susceptible to all the antimicrobials tested except for NA, a characteristic used in selecting transconjugates. *Edwardsiella ictaluri* isolates were uniformly susceptible to each of the antimicrobials tested except COL, which was used in selecting transconjugates. Each of the *E. ictaluri* isolates gave similar susceptibility patterns, which has been shown to be relatively common among isolates (Waltman and Shotts 1986).

The mating mixtures were plated onto various antibiotic-containing plates to select for transconjugates having modified resistance patterns. Transconjugates of *E. coli* 1932 had two antibiotic resistance patterns which also differed in transfer frequencies (Table 1). The pattern showing susceptibility to ROM, SXT, and CEPH but resistance to TET, CHLR, AMP, and KAN occurred at a slightly higher frequency than the one showing resistance to all of these antimicrobials.

All the *E. ictaluri* transconjugates tested gave the same antimicrobial resistance pattern but varied slightly in their transfer frequencies. *Edwardsiella ictaluri* isolates 1 and 19 received the *R*-plasmid at frequencies 100–1000 times less than the other *E. ictaluri* isolates. The resistance pattern was similar to that in *E. coli* 1932 with the exception that the resistance to CEPH was not transfered. Neither the antibiotic resistance pattern nor the frequency of transfer varied among transconjugates isolated from the different antibiotic selective plates for any *E. ictaluri* isolate. The antimicrobial susceptibility patterns of the transconjugates were retested after six serial passages and were found to be identical to those of the original transconjugate, indicating the relative stability of the *R*-plasmid received. Similar results were obtained when two other multiply-resistant *E. coli* were used as the *R*-plasmid source (data not shown).

The degree of resistance conferred by the *R*-plasmid was determined by measuring the minimum inhibitory concentration (MIC) of several antibiotics with *E. ictaluri* isolate 301 before and after acquisition of the *R*-plasmid. The change in MIC between these two organisms was found for AMP (1 μg/mL to >16 μg/mL), carbenicillin (8 μg/mL to >512 μg/mL), TET (0.25 μg/mL to 16 μg/mL), CHLR (1 μg/mL to >32 μg/mL, gentamicin (1 μg/mL to >16 μg/mL), and tobramycin (1 μg/mL to >16 μg/mL).

To determine the effect of temperature on the occurrence and frequency of *R*-plasmid transfer, matings were conducted at 20, 30, and 37°C (Table 2). The frequency of transfer from *E. coli* 1898 to *E. coli* 1932 was about 30 times more at 30°C than at 20°C and about 158 000 times more at 37°C than at 30°C. *R*-plasmid transfer from *E. coli* 1898 to *E. ictaluri* isolate 1 was similar at 20 and 30°C but was not detected at 37°C. *R*-plasmid transfer from *E. coli* 1898 to *E. ictaluri* isolate 301 was about 500 times greater at 30°C than at 20°C and about 1200 times greater at 30°C than at 37°C.

The transfer of antibiotic resistance was shown not to be due to transduction by incubating the recipient organisms with cell-

TABLE 1. Plasmid-mediated antimicrobial resistance patterns transferred from *E. coli* 1898 to recipient isolates of *E. coli* and *E. ictaluri*.

Isolate	Transferred antimicrobial resistance pattern[a]	Frequency[b]
E. coli 1932	TET, CHLR, KAN, AMP, SXT, ROM, CEPH	$10^{-7.4}$
	TET, CHLR, KAN, AMP	$10^{-6.7}$
E. ictaluri 1	TET, CHLR, KAN, AMP, SXT, ROM	$10^{-6.5}$
19	TET, CHLR, KAN, AMP, SXT, ROM	$10^{-5.6}$
301	TET, CHLR, KAN, AMP, SXT, ROM	$10^{-3.1}$
557	TET, CHLR, KAN, AMP, SXT, ROM	$10^{-3.1}$
884	TET, CHLR, KAN, AMP, SXT, ROM	$10^{-3.2}$

[a]TET, tetracycline; CHLR, chloramphenicol; KAN, kanamycin; AMP, ampicillin; SXT, sulfamethoxazole/trimethoprim; ROM, sulfamethoxine/ormetoprim; CEPH, cephalothin.

[b]Mean transfer frequency calculated from replicate mattings.

TABLE 2. Mean transfer frequency of *E. coli* 1898 to *E. coli* 1932 and *E. ictaluri* isoaltes 1 and 301 at three temperatures

	Mean transfer frequency		
Recipient	20°C	30°C	37°C
E. coli 1932	$10^{-8.9}$	$10^{-7.4}$	$10^{-2.2}$
E. ictaluri 1	$10^{-6.9}$	$10^{-6.5}$	ND[a]
E. ictaluri 301	$10^{-5.8}$	$10^{-3.1}$	$10^{-6.2}$

[a]Plasmid transfer not detected.

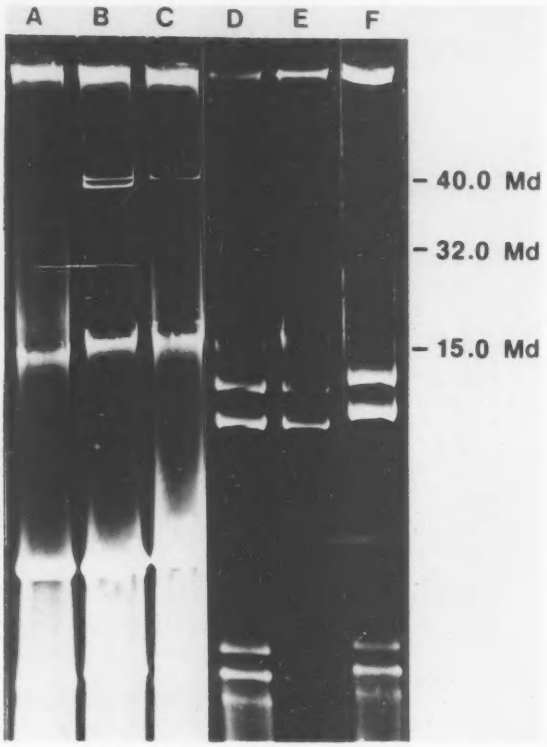

FIG. 1. Agarose gel electrophoresis of plasmid DNA from *E. coli* 1932 (lane A), *E. coli* 1898 (lane B), *E. coli* 1898/1932 transconjugate (lane C), *E. ictaluri* isolate 301 (lane D), *E. coli* 1898/*E. ictaluri* 301 transconjugate (lane E), and *E. coli* 1898/*E. ictaluri* 301 transconjugate following treatment with ethidium bromide to remove the resistance plasmid (lane F). Two plasmids (40 and 37.5 megadaltons) are demonstrated in *E. coli* 1898 (lane A), however, only the 40 megadalton plasmid is transfered to *E. coli* 1932 (lane C) and *E. ictaluri* 301 (lane E).

free supernate from cultures of the donor organism. No transductants were isolated.

A previous study had shown that *E. coli* 1898 had two plasmids which migrated at 40 and 37.5 megadaltons (Wooley et al. 1986). Gel electrophoresis demonstrated the presence of the *R*-plasmid conferring resistance in the donor isolate and subsequently in the *E. coli* 1932 and *E. ictaluri* isolate 301 transconjugates. This plasmid appears to be approximately 40 megadaltons and similar in each transconjugate. Serial passage of the transconjugate in broth containing either acridine orange or ethidium bromide by the method of Wooley et al. (1986) resulted in the loss of the *R*-plasmid within 7 d (Fig. 1).

Edwardsiella ictaluri isolate 301, which had received the *R*-plasmid, was mated with *E. coli* 1932 and *Yersinia ruckeri* isolate 11.40 to determine whether *E. ictaluri* could transfer the *R*-plasmid to other bacteria. Plasmid transfer into *E. coli* 1932 occurred at a frequency of $10^{-4.5}$ and into *Y. ruckeri* 11.40 at a frequency of $10^{-3.8}$ with conservation of the resistance pattern seen in *E. ictaluri* isolates.

Discussion

The data presented in this paper indicate the potential for *E. ictaluri* to develop plasmid-mediated antimicrobial resistance and to transfer this resistance to other bacteria. This finding is of significance since this organism is an important cause of bacterial disease and mortality in cultured channel catfish. Because an effective vaccine is not available, the only control measures consist of various management practises and antibiotic prophylaxis. Currently, only tetracycline and Romet have been cleared for use in catfish culture. Although, *E. ictaluri* have been shown to be susceptible to these antibiotics (Waltman and Shotts 1986), surveys of bacterial fish disease pathogens has revealed that the widespread use of antibiotics frequently leads to an increased prevalence of antimicrobial resistance usually mediated by *R*-plasmids (Aoki et al. 1974; Aoki et al. 1971; Aoki et al. 1977; Shotts et al. 1976).

The transfer of *R*-plasmid resistance occurred in all *E. ictaluri* isolates tested; however, the frequency of transfer varied between isolates. The frequency of *R*-plasmid transfer in *E. ictaluri* was shown to be temperature-related, with an optimum at 30°C. This may be of some consequence since almost all disease outbreaks attributed to this organism have occurred at water temperatures between 25 and 30°C, which is approximately the optimum growth temperature for *E. ictaluri*. At these temperatures, bacterial numbers would probably be at their highest and would require antimicrobial treatment if antimicrobials were not being used prophylactically. Such conditions would be conducive to the development of *R*-plasmid resistance in *E. ictaluri*.

The transfer of the *R*-plasmid from *E. ictaluri* isolate 301 to *E. coli* 1932 and to *Y. ruckeri* 11.40 demonstrates the potential for *E. ictaluri* to transfer *R*-plasmids to other bacterial species, and thus may allow for the spread of plasmid resistance in the environment.

Since the potential exists for *E. ictaluri* to become resistant to tetracycline and Romet, several precautions may be important for dealing with ESC. First, management practises such as reducing stressful conditions and preventing cross-contamination of ponds are major preventative measures which should be encouraged. Second, antibiotics should be used cautiously as prophylactics since such use may promote development of resistance. Third, *E. ictaluri* isolates should be continuously screened for the development of resistance. Finally, efforts should be increased to evaluate and clear new antimicrobials for use in fish culture and to develop effective vaccines.

Acknowledgements

The authors would like to thank Dr. Ta Chaun Hsu for his assistance and advice on this project. This study was supported by a USDA grant 10-21-RR211-048.

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Mass Flux and "Nutritional Composition" of settling Epilimnetic Particles in Lake Michigan[1]

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Gardner, W. S., B. J. Eadie, J. F. Chandler, C. C. Parrish, and J. M. Malczyk. 1989. Mass flux and "nutritional composition" of settling epilimnetic particles in Lake Michigan. Can. J. Fish. Aquat. Sci. 46: 1118–1124.

A series of sediment-trap samples, collected at a 30-m depth in southeastern Lake Michigan, was analyzed to evaluate the seasonal flux and nutritional value of settling epilimnetic particles as potential food for benthic organisms. Flux was highest in the spring (due in part to resuspension), lowest in the summer during stratification, and intermediate during autumn months. Organic content of the particles ranged from 10% ash free dry weight (AFDW) in March through May samples to 17–19% in July–August samples. During the summer, microbial degradation of organic materials occurred in the trap bottles without added preservative, as evidenced by less AFDW in nonpreserved trap bottles than in similar chloroform-preserved bottles. The percentage of AFDW occurring as lipid ranged from 3.5% in April–May up to 14% during May through August. Dominant lipid classes were hydrocarbons, polar lipids including phospholipids and chlorophyll *a*, and free fatty acids. The ratio of *n*-alkane C-17 (algal indicator) to *n*-alkane C-29 (terrestrial or resuspension indicator) and the ratio of biogenic silica to AFDW in preserved samples both reached a maximum in May, shortly before lipid content peaked in the benthic amphipod, *Pontoporeia hoyi*. This observation agrees with the hypothesis that *P. hoyi* receives much of its nutrition from the spring algal bloom.

On a analysé une série d'échantillons de sédiments recueillis à 30 m de profondeur dans le sud-est du lac Michigan, afin d'évaluer le taux saisonnier et la value nutritive du dépôt des particules de l'épilimnion, qui peuvent servir de nourriture au benthos. Le dépôt était maximal au printemps (en partie à cause de la remise en suspension), minimal durant la stratification estivale et moyen durant l'automne. La part organique des particules a varié entre 10 % du poids sec sans cendres de mars à mai, à 17–19 % en juillet et août. Durant l'été, la dégradation microbienne des matières organiques se faisait dans la bouteille d'échantillonnage sans aucun ajout, comme en témoigne le poids sec plus faible dans ces bouteilles que dans celles contenant du chloroforme. Le pourcentage de lipides dans le poids sec sans cendres a varié de 3,5 % en avril-mai à 14 % de mai à août. Les principaux types étaient de hydrocarbures, des lipides polaires comme des phospholipides et la chlorophylle *a*, ainsi que des acides gras libres. Dans les échantillons chloroformés, le ratio de n-alcane C-17 (indicateur algéen)/ n-alcane C-29 (indicateur terrestre ou de remise en suspension), ainsi que le ratio silice biogène/poids sec sans cendres étaient maximaux en mai, peu de temps avant que la teneur lipidique ne culmine chez *Pontoporeia hoyi*. Cette observation appuie l'hypothèse voulant que cet amphipode benthique reçoive une bonne partie de sa nourriture de la prolifération d'algues printanière.

Received June 29, 1988
Accepted March 9, 1989
(J9795)

Reçu le 29 juin 1988
Accepté le 9 mars 1989

Benthic invertebrates are important prey for fish in Lake Michigan and the other Laurentian Great Lakes, but the pathways of energy from primary production to the benthos have only recently been examined (e.g. Gardner et al. 1989). The dominant macroinvertebrate in the upper Great Lakes, *Pontoporeia hoyi*, is an important prey for many Great Lakes fish (Mozley and Howmiller 1977). This species is a detritivore that feeds on organic matter enriched with bacteria (Marzolf 1965). However, *Pontoporeia* growth appears to be strongly dependent on material settled from the water column following the spring diatom bloom (Cederwall 1977; Weiderholm 1973; Johnson 1970; Johnson 1988). In Lake Michigan, lipids in *P. hoyi* increase in the spring soon after the bloom (Gardner et al. 1985a; Gauvin et al. 1989; Landrum 1988). Diatom settling is the major factor causing phytoplankton removal from the epilimnion in the spring whereas zooplankton grazing is a more important factor later in the year during stratification (Scavia and Fahnenstiel 1987).

In this study, we measured seasonal changes in the sources, input rates, and nutritional value of settling particles by quantifying their mass flux and composition (ash free dry weight, organic nitrogen, biogenic silica, lipid content, and class composition). The particles were collected at a site in southeastern Lake Michigan where the bottom sediments are characterized by high populations of *P. hoyi* ($6900 \cdot m^{-2}$) and oligochaetes ($3000 \cdot m^{-2}$) (Nalepa 1987) and where lipid concentrations in *P. hoyi* have been studied (Gardner et al. 1985a; Gauvin et al. 1989; Landrum 1988). The composition of alkanes in the particles was determined to provide insights about the relative role

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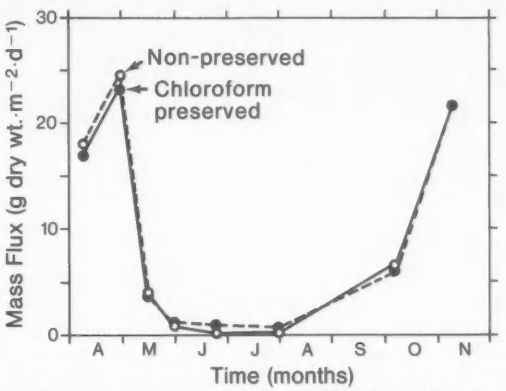

FIG. 1. Temporal mass flux of particles collected in sediment traps suspended at a depth of 30 m at a 45-m water-depth station in southeastern Lake Michigan. Data points on the *X* axis are placed at the mid-point of each sampling interval.

of autochthonous production versus terrestrial or resuspension inputs as factors supplying particles at this site.

Methods

Sediment traps (318·cm^2 collection area) were placed at a depth of 30 m at a 45-m deep station located offshore from Grand Haven, Michigan (near Station H-31; Nalepa 1987) using previously described techniques (Eadie et al. 1984). Particles were collected during the period of April 23 to November 26 at intervals ranging from about 2 wk to 2 mo as indicated in Fig. 1. For each setting, three sediment traps were suspended at the same depth. One collection bottle was untreated ("nonpreserved sample"), whereas the contents of the two others were preserved with about 25 ml of chloroform to prevent microbial activity ("preserved samples"). Mass flux and nutritional content were examined for both preserved and nonpreserved particles, to evaluate the stability of different organic fractions during trap collection. After collection, one of the preserved samples was used for hydrocarbon analysis. Samples from the other two treatments were dried for at least 3 d in a 60°C air oven, scraped out of the bottles, weighed, ground with a mortar and pestle, and stored at 4°C until analysis. Trap collections for the different treatments were not replicated, but previous studies with triplicate traps at each of four stations in Lake Michigan showed coefficients of variation in sample weights that ranged between 1 and 10% (mean 5.5%; Eadie et al. 1984).

A measured portion of the dried samples was ashed at 550°C for 1 h to determine ash free dry weight (AFDW) that was assumed to represent total organic matter. "Organic" (Kjeldahl) nitrogen was measured after it was converted to ammonium by digestion in an aqueous mixture of 20% concentrated H_2SO_4, 6.7% K_2SO_4 and 0.8% HgO (at 200°C for 1 h and at 360°C for 25 min). This procedure was modified from Technicon Industrial Method No. 327-74 (September 1974). The digestate was diluted to original volume (20 ml) and 100 μL of the solution was diluted with 10 ml of citric acid buffer (0.5% citric acid adjusted to pH 9.5 with NaOH) and analyzed fluorometrically for ammonium (Gardner 1978). Biogenic silica was extracted and analyzed by auto analyzer using Technicon components (Krausse et al. 1983). Lipids were extracted and analyzed gravimetrically using micro techniques (Gardner et al. 1985b). A measured portion (10–20 μL) of each gravimetric extract was sealed under nitrogen and stored frozen until analysis by thin-layer chromatography with flame ionization detection (TLC-FID; Parrish 1987a; Parrish et al. 1988). The lipid classes in 2–4 different extracts from each trap sample was separated by Chromarod TLC. The precision (SD) of the TLC-FID determination of each lipid class in four separate extracts was, on average, ± 5% of the sum of the lipid classes. Separate chloroform-treated samples were Soxhlet-extracted overnight in acetone/hexane (1:2) for alkane analysis by gas chromatography. Extract volumes were reduced to less than 1 mL, then eluted with 12 mL pentane from a 0.6 × 10 mm column of activated silica gel and dried under a stream of nitrogen to 1 mL. They were analyzed on an HP 5890 gas chromatograph equipped with a 25 M BP-1 capillary column and flame ionization detector.

Results and Discussion

As noted in previous Lake Michigan studies (Eadie et al. 1984), mass flux was much higher in spring and autumn during holomixis than in late summer when the lake was stratified (Fig. 1). This difference can be attributed to the downward flux of resuspended particles during holomixis and to the production of diatoms produced in response to the mixing of nutrients into the photic zone (Eadie et al. 1984). During the stratified period, particles and nutrients from the hypolimnion are not substantially resuspended into the epilimnion except during periodic upwellings (Mortimer 1971; Bell and Eadie 1983). The nutritional value of organic matter associated with resuspended particles may be low because much of the available nutrients would likely have been removed from them by previous grazers, predators, or bacteria.

Composition of Trap Material

Seasonal compositional changes were observed for all of the organic constituents. Relative to total dry weight, organic components (AFDW), Kjeldahl nitrogen, biogenic silica, and total lipid) were higher in the late spring and in summer than during spring and autumn (Fig. 2). Expressed as a ratio to total organic matter (AFDW), organic nitrogen and biogenic silica underwent only moderate changes in composition during the sampling period, but total lipid concentration more than tripled in preserved samples during the interval of 7 May to 9 June compared with values in the early spring (Fig. 3). This selective increase in lipids may be important in energy fluxes as lipids tend to be transferred to consumers more efficiently than other classes of organic materials and are also readily stored by invertebrates (Lee 1975).

Unique treatment-specific patterns were observed for different constituents. The AFDW concentrations in nonpreserved samples were similar to those in the chloroform-preserved ones during spring and autumn but were consistently lower in the summer months (Fig. 2). The composition of particles actually reaching the benthos is probably intermediate between that of the two treatments as some decomposition and grazing of particles would occur during the time that the particles are settling the rest of the way to the bottom. Epilimnetic decomposition processes are likely enhanced by relatively high temperatures in the summer. In addition, the ratio of newly-formed "labile" particles to old-resuspended "refractory" particles would likely

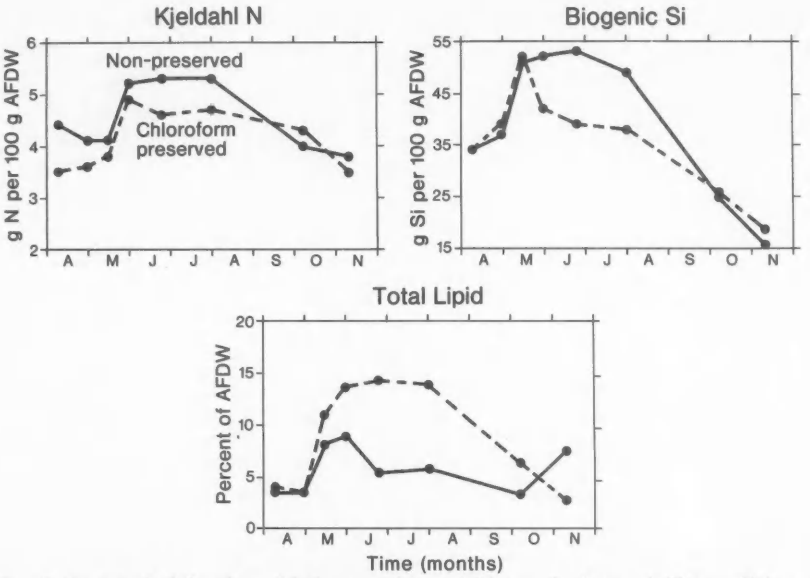

FIG. 2. Content of ash free dry weight (percent of dry weight), organic nitrogen (milligrams N (grams dry wt)$^{-1}$), biogenic silica (milligrams Si (grams dry wt)$^{-1}$), and total lipid (percent of dry weight) in sediment-trap materials collected at intervals over the period of March 27 to November 26, 1986. The nonpreserved sample of October–November was contaminated with dead fish.

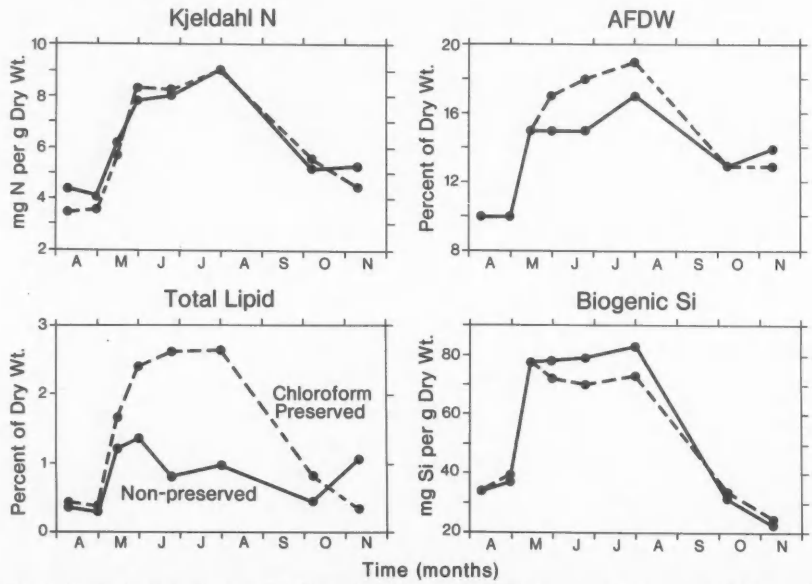

FIG. 3. Content of organic nitrogen, biogenic silica, and total lipid expressed as percent relative to ash free dry weight in sediment-trap materials collected at intervals over the period of March 27 to November 26, 1986. The nonpreserved October–November sample was contaminated with dead fish.

be higher during summer stratification (Eadie et al. 1984). Kjeldahl nitrogen and biogenic silica concentrations were not dramatically different in the two fractions throughout the season, but were higher, relative to AFDW, in the nonpreserved samples than in the preserved ones during summer (Fig. 3). This result may have been due in part to selective degradation of organic compounds containing low levels of nitrogen and biogenic silica (e.g. lipids and carbohydrates). Of all the components that we examined, lipids showed the most evidence of degradation. Except for one untreated sample (October–November) that was contaminated with dead fish, lipids were consistently lower in the nonpreserved samples than in the preserved ones, especially during June and July (Figs. 2 and 3). Also, portions of the mineralized nitrogen and silica were prob-

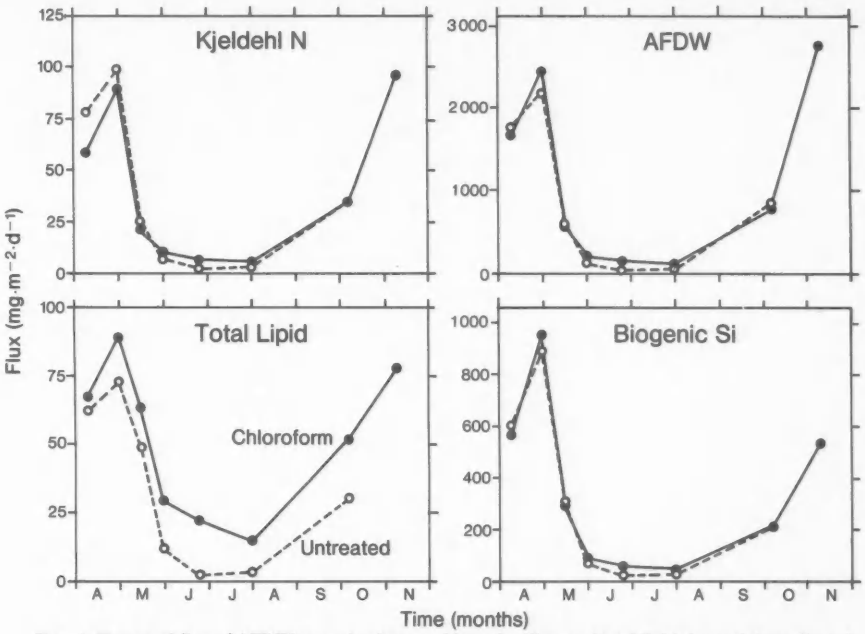

FIG. 4. Temporal flux of AFDW, organic nitrogen, biogenic silica, and total lipids in particles collected in sediment traps suspended at a depth of 30 m at a 45-m water-depth station in southeastern Lake Michigan. Dots represent nonpreserved samples. Circles represent preserved samples.

ably measured along with the organic forms in the collected samples. In contrast, CO_2 from mineralized carbon would likely be lost when the samples were dried.

Nutrient Flux

The nutritional value of incoming particles as food to benthic and hypolimnetic organisms depends on both the quantity and composition of the particles. The flux of individual constituents was calculated for each sampling period (Fig. 4). Seasonal changes in mass flux (Fig. 1) appear to be quantitatively more important than changes in relative composition (e.g. AFDW, Kjeldahl nitrogen, biogenic silica, or total lipid; Figs. 2 and 3) in determining the rate of delivery of nutrients to the bottom of the lake. This interpretation is complicated somewhat by the fact that resuspended material, dominant in the spring and fall, has lower nutritional content than new material because it contains relatively refractory materials. However, nutritionally-rich large diatoms, that sink quite rapidly, are also most abundant in the spring (Scavia and Fahnenstiel 1987).

Lipid Class Composition and Flux

The lipids in the sediment-trap material were separated into as many as nine different classes by Chromarod TLC. Total aliphatic hydrocarbons (HC), free fatty acids (FFA), sterols (ST), acetone-mobile polar lipids (AMPL), and phospholipids (PL) were present in all extracts (Fig. 5). Small concentrations of diglycerides, hydrolysis products from triglycerides (TG) and PL, were detected in only two nonpreserved samples collected late in the season (data not shown). A small sterol ester/wax ester (SE/WE) peak occurred in the April–May trap sample that was collected soon after the spring diatom bloom. The copepods, *Limnocalanus macrurus* and *Senecella calanoides*, are likely contributors of WE to this peak (Chandler et al. 1989). Although the trend was not dramatic, ST and AMPL appeared to be proportionally highest in early spring when the diatoms were likely dividing most rapidly. In agreement with this tentative finding, concentrations of ST and AMPL were linearly correlated with growth rate in a marine diatom (Parrish and Wangersky 1987). Sterols are an important component of membranes, while AMPL in diatoms consist of pigments and glycolipids (important lipids in chloroplasts; Parrish et al. 1988). A large peak for total aliphatic hydrocarbons (HC) always occurred in chromatograms of preserved sediment-trap materials. On average, HC accounted for 28% of the TLC-FID-measured total lipids in the eight traps. A high content of HC seems to be characteristic of sediment-trap material from the Great Lakes (Parrish 1987a; C. C. Parrish, unpubl. data). The nature and source of the majority of these aliphatic hydrocarbons are unknown, but they appear to be quite labile. On average, HC constituted only about 10% of the total lipids in nonpreserved sediment-trap samples.

The highest proportion of TG, $8.3 \pm 0.2\%$, was measured in the preserved sediment-trap lipids collected during mid-May. This peak in TG relative to other lipid classes may reflect a significant input of diatom storage material to the sediment trap. It is coincident with a large peak in the n-C_{17} to n-C_{29} alkane ratio (Figs. 6 and 7) and with a peak in the ratio of biogenic silica to AFDW (Fig. 3) in the preserved samples. The fact that TG was never the major lipid class in sample extracts suggests that the settling diatoms were likely not under severe nutrient stress, as TG are often the major lipid component in nutrient-limited diatoms (Parrish 1987b; Parrish and Wangersky 1987; S. Kilham, University of Michigan, unpubl. data). Even when HC concentrations were subtracted from the sum of lipid-class concentrations, TG accounted for less than 15% of the

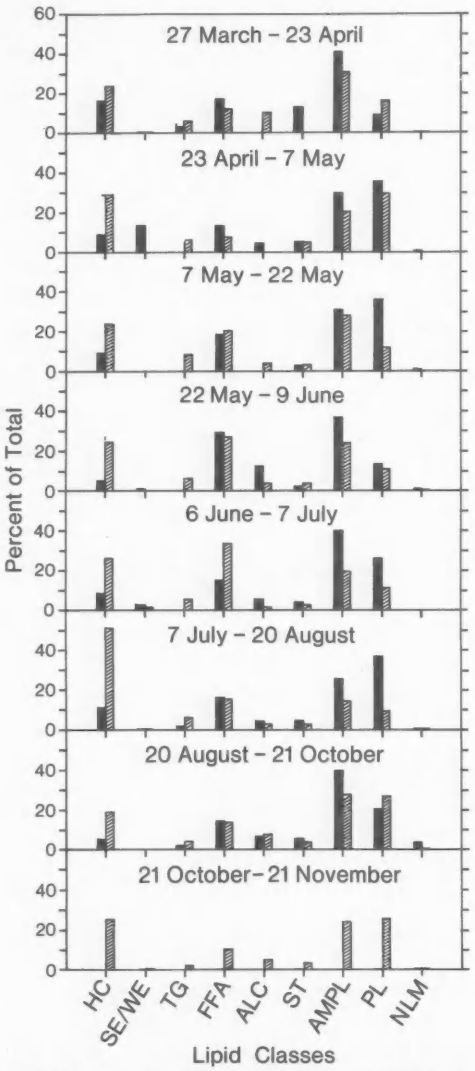

FIG. 5. Relative composition (percent of all classes) of lipid classes in preserved (hatched bars) and nonpreserved (dark bars) seasonal sediment-trap collections. HC = hydrocarbons; SE/WE = sterol esters/wax esters; TG = triglycerides; FFA = free fatty acids; ALC = free aliphatic alcohols; ST = sterols, AMPL = "acetone-mobile polar lipids," including chlorophyll; PL = polar lipids; NML = "nonlipid material."

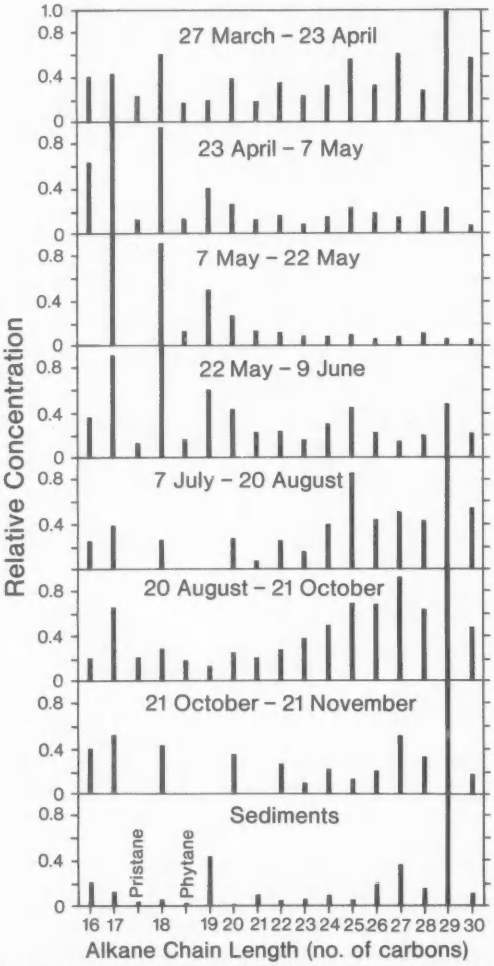

FIG. 6. Normal alkane composition of preserved trap materials (and local surface sediment) for the periods indicated. Short chain lengths are indicators of plankton. Long chain lengths indicate material of terrestrial origin.

remaining oxygen-containing lipids in any of the trap samples. Likewise, TG accounted for less than 15% of the lipids in diatoms growing in nutrient replete media (Parrish and Wangersky 1987). Like HC, TG were relatively lower in the nonpreserved sediment-trap material than in the preserved samples. The absence of large increases in FFA with the removal of TG and other lipids in the nonpreserved sediment-trap lipids suggests that the TG in the nonpreserved samples were consumed rather than simply hydrolyzed.

Free fatty acids accounted for about 18% of the lipids, on average, both in the eight treated and in the seven nonpreserved samples. The largest FFA percentages occurred during the summer. Hydrolysis of acyl lipids would be greatest during this period due to the relatively high temperatures.

The HC content of the preserved sediment trap material increased each month until it peaked at 13.3 $mg \cdot g^{-1}$ at the end of the summer (July/August trap; Fig. 5). This value is similar to solvent-extractable HC values of 7.4–11.9 $mg \cdot g^{-1}$ in preserved 35-m deep sediment-trap samples collected during the summer of 1978 from four locations in southeastern Lake Michigan (Meyers et al. 1984). However, our HC fluxes of >5.8 $mg \cdot m^{-2} \cdot d^{-1}$ were much higher than the "total aliphatic hydrocarbon" flux of 0.074 $mg \cdot m^{-2} \cdot d^{-1}$ previously measured at 35 m (Meyers et al. 1980). This difference may be due to considerable spatial or temporal variation in hydrocrbon fluxes or to differences in hydrocarbon measurement techniques.

Fatty acids are the major component, by mass, of the TG, FFA, and PL peaks. Sums of these "acyl lipid components" are therefore comparable with the "fatty acid" content previously estimated by analyzing fatty acid methyl esters prepared

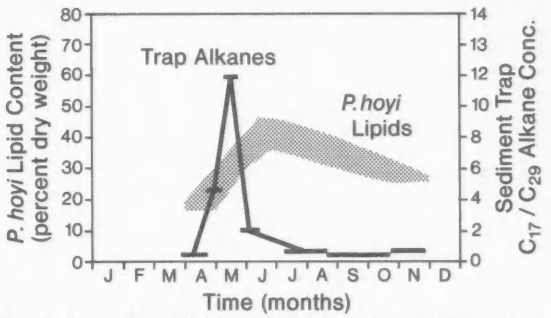
Trap Alkanes
P.hoyi Lipids
P. hoyi Lipid Content (percent dry weight)
Sediment Trap C17 / C29 Alkane Conc.
Time (months)
J F M A M J J A S O N D
0 10 20 30 40 50 60 70 80
0 2 4 6 8 10 12 13 14

FIG. 7. Seasonal total lipid content of *P. hoyi* averaged over 2 yr of collection (data taken from Gardner et al. 1985; Landrum 1988; Gauvin 1989). Also shown is the ratio of the planktonic *n*-alkane C_{17} to the terrestrial *n*-alkane C_{29}. The April–June peak is a clear signal of autochthonous carbon input. The sediment value for this ratio is 0.2.

from solvent extracts (Meyers et al. 1980, 1984). The acyl lipid content of our preserved sediment-trap material increased each month until mid-summer when it peaked at 13 $mg \cdot g^{-1}$. This value is within the range of 0.9–49 $mg \cdot g^{-1}$ given for fatty acid content of sediment-trap material collected between 30 and 40 m during the summer of 1978 (Meyers et al. 1984). The acyl lipid flux measured in June–July was 11 $mg \cdot m^{-2} \cdot d^{-1}$ as compared with 4.6 $mg \cdot m^{-2} \cdot d^{-1}$ in July–August. These two values bracket summer (1978) values for total fatty-acid flux measured in 35-m (4.9 $mg \cdot m^{-2} \cdot d^{-1}$) and 73-m (7.9 $mg \cdot m^{-2} \cdot d^{-1}$) sediment traps (Meyers et al. 1980).

Our ALC value of 0.42 $mg \cdot g^{-1}$ in June–July is comparable with 0.22 $mg \cdot g^{-1}$ previously observed at 40-m depth in southern Lake Michigan, but is much lower than the values of 5 to 60 $mg \cdot g^{-1}$ reported for traps deployed between 31 and 35 m (Meyers et al. 1984)). The concentration of ST, the cyclic aliphatic alcohols, in our June–July trap was 0.71 $mg \cdot g^{-1}$ as compared with the range of 0.1 to 2.1 $mg \cdot g^{-1}$ for traps deployed between 30 and 40 m (Meyers et al. 1984).

Evidence for Direct Pelagic–Benthic Energy Coupling

Measuring the alkane composition of particles by gas chromatography provides information about possible sources of the material. For example, *n*-alkanes with chain length C_{23} to C_{33} are strong indicators of a terrestrial source, while chain lengths of C_{12} to C_{20} characterize aquatic plankton (Simoneit 1978; Muller 1987; Thurman 1985). Among these hydrocarbons, C_{17} is particularly useful as a plankton indicator. The three trap collections covering the period 23 April through 9 June had a predominance of autochthonous derived alkanes (Fig. 6). This "phytoplankton signal" agrees with the peak in biogenic silica flux observed in the preserved sample (Fig. 3) and supports other evidence that material collected in traps during this period were predominantly algae (diatoms; Scavia and Fahnenstiel 1987). In contrast, early in the spring and again in late summer–fall, the alkane distribution in the trapped material had a substantial terrestrial component. Terrestrial hydrocarbons are relatively refractory in the lake (Meyers et al. 1984), as indicated by the alkane distribution in the surface sediment from this station (Fig. 6). Thus, these trapped hydrocarbons can be accounted for by both resuspension of surface sediments and fresh terrestrial input from the relatively large (average 44 $\cdot m^{-3} \cdot s^{-1}$ flow) nearby Grand River.

The timing of lipid increases in *P. hoyi* appears to follow that of the spring diatom bloom as suggested previously (Gardner et al. 1985a, 1989). Lipids in *P. hoyi* increased dramatically at this site during the interval of April to June and then gradually decreased during the rest of the season (Gardner et al. 1985a; Gauvin et al. 1989; Landrum 1988). The lipid increase in *P. hoyi* occurred directly after the peak in the *n*-alkanes C_{17} (planktonic): C_{29} (terrestrial) ratio (Fig. 7). As summer approached, the rate of delivery of "labile" materials decreased substantially and the *P. hoyi* appeared to gradually use up the stored lipids that were assimilated in the spring. As mentioned above, the large size and high density of spring diatoms such as *Melosira* likely cause them to sink to the benthos more readily than smaller phytoplankton (e.g. flagellates and green and bluegreen algae) that occur later in the season and are more heavily grazed by pelagic zooplankton than *Melosira* (Scavia and Fahnenstiel 1987; Fahnenstiel and Scavia 1987).

The alkane ratios (Fig. 7) and biogenic silica to AFDW ratios (Fig. 3) in the preserved samples provided a much stronger signal of nutritional inputs, that appeared to be related to lipid increases in *P. hoyi*, than did the other "chemical indicators" (Figs. 2–4). In contrast to the C_{17}:C_{29} ratios and the biogenic silica:AFDW ratios in the preserved samplcs, that showed a sharp peak in May, concentrations of AFDW, Kjeldahl nitrogen, and total lipid, remained relatively high and consistent relative to dry weight (or AFDW) during the period of May through August (Figs. 2 and 3).

The high concentrations of lipids in preserved samples during the summer caused the total lipid flux to vary less seasonally than the fluxes of the other components (Fig. 4). Despite the continuing high lipid levels of the preserved trap particles (Figs. 2 and 3) and the relatively high flux of total lipids throughout the season (Fig. 4, the lipid concentrations in *P. hoyi* still decreased from June through August (e.g. Fig. 7). Similarly, *P. hoyi* growth rates are highest in the spring and decrease as the season progresses (e.g. in South Bay, Lake Huron, Johnson 1988). Thus, although the previously suggested relationship between the spring diatom bloom and lipid increases in *P. hoyi* (Gardner et al. 1985a, 1988) is implied by the alkane ratios and biogenic silica concentrations and fluxes, the relationship between total lipids in incoming food and *P. hoyi* lipid assimilation is not as clear. It is possible that *P. hoyi* may not assimilate its storage lipids completely from food lipids as occurs for *Daphnia* (Goulden and Place 1987). Preliminary *P. hoyi* feeding studies (M. Quigley, Great Lakes Environmental Research Laboratory, unpubl. data) indicate that lipid accumulation or loss in *P. hoyi* may not be exclusively related to the lipid content of the food.

To consider whether dietary lipids in the incoming trap materials were quantitatively sufficient to account for the lipid increases in *P. hoyi*, we compared the total lipid flux in settling particles during spring to lipid increases in *P. hoyi*. Based on the data from Fig. 4, the integrated total lipid flux during the period 27 March to 9 June was 4.6 g $lipid \cdot m^{-2}$. This flux compared with total lipid increases in *P. hoyi* of 2.1 g $lipid \cdot m^{-2}$ (based on a *P. hoyi* biomass of 5.3 g $AFDW \cdot m^{-2}$ at the 45-m station (Nalepa et al. 1985), a Ponar grab correction factor of 1.5 (Nalepa et al. 1989), a DW:AFDW conversion factor of 1.1 (Gardner et al. 1985a), and a *P. hoyi*-lipid increase of 24% of DW (Fig. 7)). If our assumptions are reasonably valid, this comparison suggests that incoming lipid supply would be adequate to account for the lipid increases in *P. hoyi* only if a substantial portion of the total lipids in the settling materials

were ingested and efficiently assimilated by *P. hoyi*. However, near-quantitative assimilation of the lipids passing through a 30-m depth by *P. hoyi* seems unlikely because a sizable portion of the lipids may be degraded before reaching the bottom and because portions of the remaining lipids are likely nutritionally unavailable to *P. hoyi* (e.g. refractory hydrocarbons; Meyers et al. 1984) or not readily used for energy storage (e.g. hydrocarbons and pigments) in the animal. An alternative explanation is that *P. hoyi* may synthesize a portion of its lipids from other materials such as carbohydrates. We did not analyze sediment-trap particles for carbohydrates, but proteins (= Kjeldahl nitrogen × 6.26) and lipids accounted for only 25 to 44% of the total AFDW (based on preserved-sample data of Fig. 3). If "available", carbohydrate constitues a large portion of the undefined AFDW, particularly in spring diatoms, it may partially account for the *P. hoyi* lipid increases that occur after the spring bloom.

Acknowledgements

We thank J. Bell for assembling and setting the sediment traps, M. Quigley for helpful discussions on *P. hoyi* nutrition, W. Burns, J. Grimes, and D. Morse for helping collect trap samples, and G. Fahnenstiel, P. Landrum, T. Nalepa, and M. Quigley for reviewing the manuscript.

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Effects of Food Removal on Nutrient Release Rates and Lipid Content of Lake Michigan Pontoporeia hoyi[1]

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Gauvin, J. M., W. S. Gardner, and M. A. Quigley. 1989. Effects of food removal on nutrient release rates and lipid content of Lake Michigan *Pontoporeia hoyi*. Can. J. Fish. Aquat. Sci. 46: 1125–1130.

Pontoporeia hoyi, the dominant benthic invertebrate in the upper Great Lakes, appears to be well adapted to environments with seasonal inputs of high-quality food such as those supplied by the spring diatom bloom. Ammonium and phosphate excretion rates and lipid content were examined in *P. hoyi* under the following conditions: (1) field animals sampled seasonally, (2) food-deprived animals in filtered lake water, and (3) control animals held in native sediments without new food inputs. Nutrient excretion rates for *P. hoyi* (0.5 to 2 nmole NH_4 (milligrams dry weight)$^{-1}\cdot h^{-1}$ and 0 to 0.15 nmole PO_4 (milligrams dry weight)$^{-1}\cdot h^{-1}$) were low relative to rates previously reported for other benthic and pelagic invertebrates and varied little among the three treatment groups. In contrast to the animals held in the laboratory without new food inputs, field-collected *P. hoyi* accumulated increased levels of lipids following the spring diatom bloom. However, after lipid levels in field animals peaked in May, they decreased during the rest of the season at rates similar to those of starved and control animals. *P. hoyi*'s low nutrient-excretion rates, and its ability to accumulate and store energy as lipids, for periods when food is not abundant, appear to be important factors allowing this animal to thrive in temperate lakes with spring diatom blooms.

Pontoporeia hoyi, principal invertébré benthique des Grands Lacs d'amont, semble bien adapté aux milieux qui reçoivent des apports saisonniers d'aliments de grande qualité, tel celui fourni par la prolifération printanière de diatomées. On a mesuré les taux d'excrétion des ions ammonium et phosphate et les teneurs lipidiques chez des spécimens : (1) recueillis chaque saison sur le terrain; (2) privés de nourriture dans de l'eau de lac filtrée; (3) témoins conservés dans les sédiments d'origine sans nouvel apport alimentaire. Les taux d'excrétion (0,5 à 2 nmoles $NH_4\cdot$mg de poids sec$^{-1}\cdot h^{-1}$ et 0 à 0,15 nmole $PO_4\cdot$mg de poids sec$^{-1}\cdot h^{-1}$) étaient faibles par rapport aux taux mesurés chez d'autres invertébrés pélagiques ou benthiques, et ont peu varié parmi les trois groupes étudiés. Contrairement aux animaux soumis au jeûne en laboratoire, les spécimens récoltés sur le terrain avaient accumulé des réserves de lipides après la prolifération des diatomées. Cependant, après avoir culminé en mai, les teneurs en lipides diminuaient durant le reste de la saison à un rythme semblable à celui des témoins et des spécimens soumis au jeûne. Le taux faible d'excrétion d'éléments nutritifs chez *P. hoyi*, ainsi que son aptitude à accumuler des réserves lipidiques pour les périodes de disette, semblent être des facteurs importants de la survie de cet animal en lacs tempérés sujets aux prolifération de diatomées.

Received June 29, 1988
Accepted March 9, 1989
(J9794)

Reçu le 29 juin 1988
Accepté le 9 mars 1989

The amphipod, *Pontoporeia hoyi* (previously called *Pontoporeia affinis*), is an important benthic animal in the Great Lakes. It constitutes up to 88% of the zoobenthic biomass in the upper Great Lakes (Cook and Johnson 1974) and can reach densities of 34 000 individuals$\cdot m^{-2}$ (Nalepa et al. 1985). *Pontoporeia hoyi* is eaten by a wide variety of fishes (Wells 1980) and may affect contaminant transfer dynamics in the lakes (Nalepa and Landrum 1988). Lipophilic toxins such as polychlorinated biphenyls and polycyclic aromatic hydrocarbons are bioaccumulated by *P. hoyi* to levels much greater than occur in surrounding sediments (Eadie et al. 1983; Landrum 1983; Evans et al. 1982). *Pontoporeia hoyi* also mineralizes nutrients in Great Lake sediments; excretion by *P. hoyi* appears to account for a significant fraction of total sediment–water nitrogen flux at some sites in Lake Michigan (Gardner et al. 1987).

In contrast to most other known amphipods, *P. hoyi* feeds intermittently and appears to eat more frequently (as evidenced by gut fullness) in the spring than during other times of the year (Quigley 1988). *Pontoporeia hoyi* growth rates (Johnson and Brinkhurst 1971; Johnson 1988) and lipid levels (Gardner et al. 1985b; Landrum 1988) increase following spring phytoplankton blooms and then decrease during late summer and winter. Diatoms are the dominant phytoplankton component in Lake Michigan in the spring, when abundant silica is available, but decrease as the season progresses (Fahnenstiel and Scavia 1987). Sinking of large diatoms (e.g. *Melosira* spp.) is the major factor causing losses of phytoplankton from the epilimnion during the spring, whereas later in the season, zooplankton grazing removes most of the diatoms from the water (Scavia and Fahnenstiel 1987). Thus, large diatoms of high nutritional quality may be available to *P. hoyi* for a period in the spring,

[1]GLERL contribution No. 625. Most of this work was conducted at GLERL as part of J.M. Gauvin's research for an M.Sc. degree at Eastern Michigan University, Ypsilanti, MI, USA.

but not during the rest of the year when the nutritional quality of available particles may be lower (Gardner 1989a, 1989b).

We suspected that the availability of this high-quality food in the spring may allow *P. hoyi* to accumulate lipid reserves that, in turn, would support its metabolic needs during later months when nutrient inputs are low. For example, cold water arctic zooplankton, that experience sporadic food supplies and accumulate high lipid levels, can survive months without food (e.g. Lee and Barnes 1975). For this scenario to be feasible for *P. hoyi*, its metabolic rates would have to be sufficiently low to allow it to survive for long periods without depletion of critical reserves of energy and nutrients. Low temperatures may be essential for long survival time, as warm water pelagic invertebrates such as *Daphnia* (Holm and Shirpiro 1984) and the subtropical marine copepod *Eucalanus pileatus* (Gardner and Paffenhöfer 1982) can survive only for periods of days/weeks without food.

To gain insights into *P. hoyi*'s physiological and biochemical characteristics that allow it to thrive in oligotrophic, temperate environments such as the upper Great Lakes, we examined *P. hoyi*'s potential longevity and measured its ammonium and phosphate excretion rates and its lipid content under the following conditions: (1) field animals sampled seasonally, (2) group and individual food-deprived animals in filtered lake water, and (3) control animals held in native sediments (without new food inputs) for several months after collection.

Methods

Collection and Experimental Handling of *Pontoporeia*

Pontoporeia hoyi samples were collected in southeastern Lake Michigan at a site 9.5 km southwest of Grand Haven, Michigan, where the water depth is 45 m. This site had a mean *P. hoyi* density of about 6900 individuals·m^{-2} (Nalepa 1987). Sediment samples containing *P. hoyi* were taken with a PONAR grab, placed in a cooler containing hypolimnetic water, packed in ice, and transported to the laboratory in Ann Arbor, Michigan. Animals were stored with sediments in the laboratory at 4°C until they were analyzed within 6 d of collection.

Samples were collected from the lake on April 23, May 7, May 22, July 8, August 20, November 26, and December 8, 1986. Animals for the long-term laboratory studies were collected on April 23, 1986. On April 29, the animals were divided into "control" (those incubated in native sediments) and "starved" groups. Each group consisted of approximately 250 animals placed in a 24 cm long × 15 cm × 15 cm glass aquarium (to give a density of 7000 animals·m^{-2}) that was about three-fourths filled with cold (ca 4°C) lake water from the collection site. For the control group, freshly collected sediment from 0–4 cm depth at the same site was sieved through a 250-μm mesh size screen to remove invertebrates, so a known density of animals could be added. The sieved sediment was added to the aquarium to a depth of 3 cm. For the starved group, no sediments were placed in the aquarium and the lake water was first filtered through a Gelman AE glass fiber filter (1 μm retention) to remove particles and associated nutrients. The water surfaces of both aquaria were covered with a layer of plastic Saran Wrap to prevent animals from being trapped at the air–water surface but yet allow oxygen to diffuse into the water. The animals incubated as a group without added food may not have been completely starved because they likely received some nutrition from the remains of animals that died during the experiments. For comparison, lipid concentrations were also determined on a second set of animals (sampled in December, 1986) that were incubated individually in scintillation vials, for up to 40 d, in oxygen-saturated filtered lake water over a substrate of ashed, coarse sand.

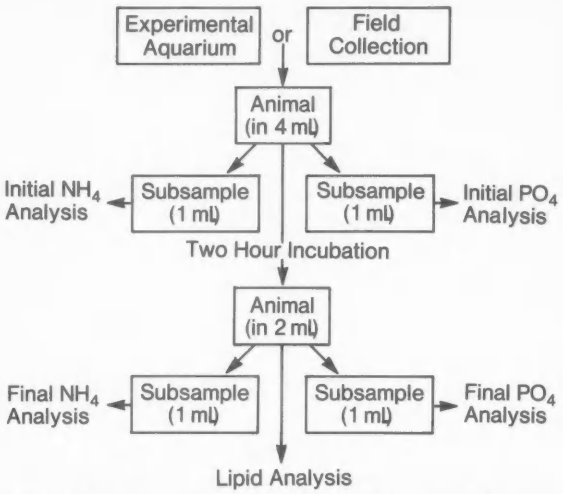

FIG. 1. Outline of incubation and nutrient sampling scheme for measuring nutrient excretion and lipid content on individual *Pontoporeia hoyi*.

Analytical Measurements

Ammonium and phosphate excretion rates, dry weight, and lipid content were successively determined on the same animals (see schematic flow diagram, Fig. 1). Mean *P hoyi* dry weights on the different sampling dates ranged from 1.2 to 2.2 mg·individual^{-1} for field animals, 0.8 to 1.1 mg·individual^{-1} for starved animals, and 0.9 to 1.3 mg·individual^{-1} for control animals. Ammonium and phosphate release rates were determined after 2-h incubations at 4°C under dim light in low-nutrient media (double strength NOPN, Lehman 1980). Before incubations, individual animals were gently removed from sediments and placed in a beaker of filtered lake water to remove associated debris. Each of 5 to 10 animals from each group was placed separately into its own 5-ml polyethylene vial containing 4 ml of low-nutrient water. The water was then gently mixed and 2 ml were immediately drawn off with a clean plastic syringe for initial NH_4 and PO_4 measurements. The initial samples were stored in clean, capped vials until the end of incubations. The animals were incubated in the remaining 2.0 ml of water for 2.0 h to allow accumulation of nutrients for excretion-rate measurements. After incubation, the contents of each vial were gently stirred and most of the incubation water was removed for final nutrient measurements. Ammonium was measured with a micro-fluorometric technique (Gardner 1978) and phosphate with a discrete-injection autoanalyzer method (Gardner and Malczyk 1983).

Immediately following incubations, the animals from the excretion experiments were placed in individual tared glass tubes and dried at 55°C for 4 d in preparation for lipid analysis. Lipid content was determined on each animal with a microgravimetric procedure (Gardner et al. 1985a). Occasional outlying data points for any group of animals were rejected by the

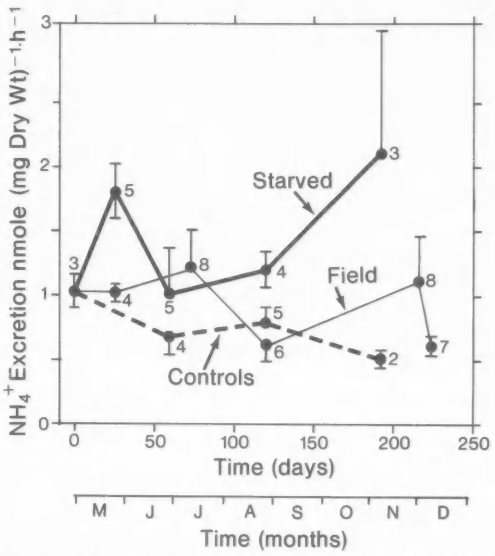

FIG. 2. Ammonium release rates of experimental and field *Pontoporeia hoyi* collected at various times in 1986. The numbers adjacent to the data points indicate the number of animals analyzed to obtain the mean. Range bars are SE.

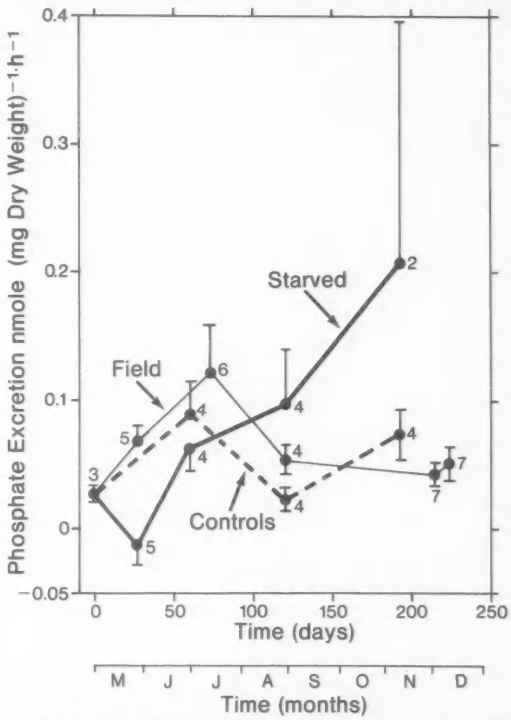

FIG. 3. Phosphorus release rates of experimental and field *Pontoporeia hoyi* collected at various times in 1986. The numbers adjacent to the data points indicate the number of animals analyzed to obtain the mean. Range bars are SE.

Q test (Skoog and West 1963) and were not used in calculations of means.

Results

Ammonium Release

Except for the last measurements on starved animals on Day 191, mean *P. hoyi* ammonium release rates ranged between 0.5 and 1.8 nmole (milligrams dry weight)$^{-1}$·h^{-1} (Fig. 2). When the data sets for field-collected animals were combined, the ammonium excretion rates correlated significantly ($P = 0.02$) to the dry weights of the animals ($r = -0.374$, $n = 38$) but this relationship accounted for only a small portion (14%) of the total variance in excretion rates. Although ammonium excretion rates were remarkably consistent throughout the experiment (Fig. 2), the rates were lower than ash free dry weight (AFDW) specific excretion rate values previously reported for *P. hoyi* in Lake Michigan at either 4°C (3–5 nmole (milligrams ash free dry weight)$^{-1}$·h^{-1}) or 11°C (6 nmole (milligrams ash free dry weight)$^{-1}$·h^{-1}) (Gardner et al. 1987). A small part (10–20%) of the apparent discrepancy between the two studies at 4°C was caused by the use of dry weight versus AFDW. The remaining differences may have been partially caused by a variation in the delay between sampling and excretion measurements (see discussion below). Both data sets indicated that excretion rates for *P. hoyi* at 4–11°C tend to be comparable or lower than those for other Lake Michigan macroinvertebrates held at higher temperatures (4.5 and 7.8 nmole NH_4 (milligrams dry weight)$^{-1}$·h^{-1} for chironomids and 9.2 and 4.8 nmole NH_4 (milligrams dry weight)$^{-1}$·h^{-1} for tubificids at 12° and 22°C, respectively Gardner et al. 1983). The excretion rates at 4°C overlapped with rates for oligochaetes (1–4 nmole NH_4 (milligrams AFDW)$^{-1}$·h^{-1}) held at the same temperature (Gardner et al. 1987) and were within a range of rates observed for a variety of benthic species in Danish coastal waters (0.3 to 3.0 nmole NH_4 (milligrams dry weight)$^{-1}$·h^{-1} (= averaged results from a variety of measurements taken over the temperature range of 3.5 to 13.6°C; Blackburn and Henriksen 1983), based on an assumed wet weight:dry weight ratio of 10). Excretion rates for all of these benthic invertebrates were much lower than those reported for warm water pelagic invertebrates (e.g. 35 to 82 nmole NH_4 (milligrams dry weight)$^{-1}$·h^{-1} for *Daphnia* spp. at temperatures of 20–25°C; Lehman 1980; Vanderploeg et al. 1986).

Phosphorus Release

Phosphorus release rates among the different groups were less than 0.15 nmole PO_4 (milligrams dry weight)$^{-1}$·h^{-1} throughout most of the experiment (Fig. 3) and resembled previous results for *P. hoyi* from Lake Michigan (0.08–0.11 nmole P (mg AFDW)$^{-1}$·h^{-1}; Nalepa et al. 1983). Phosphorus release rates were not significantly correlated with dry weights ($r = -0.231$ ($n = 32$) for field-sampled animals). As was true for ammonium, phosphorus excretion rates by *P. hoyi* were low relative to those of other benthic invertebrates in Lake Michigan (0.12 to 0.81 nmole PO_4 (milligrams dry weight)$^{-1}$·h^{-1} for *Stylodrilus*, tubificids and chironomids incubated at 5 and 20°C; Gardner et al. 1981; Nalepa et al. 1983). They were also on the low end of the range reported for various benthic invertebrates from Danish coastal sediments (0.06 to 0.7 nmole PO_4 (milligrams dry weight)$^{-1}$·h^{-1}; Blackburn and Henriksen 1983) and much lower than rates reported for *Daphnia pulex* held at 20°C (5.0 to 7.4 nmole PO_4 (milligrams dry weight)$^{-1}$·h^{-1}; Lehman 1980).

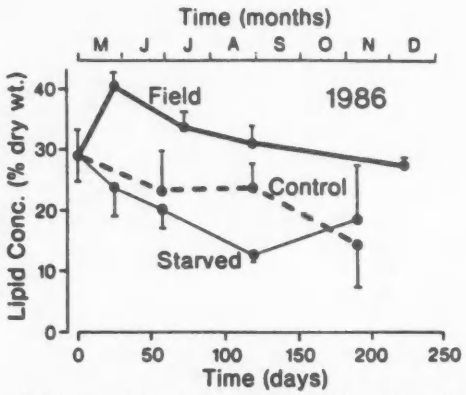

FIG. 4. Lipid content of experimental and field *Pontoporeia hoyi* collected at various times in 1986. Range bars are SE.

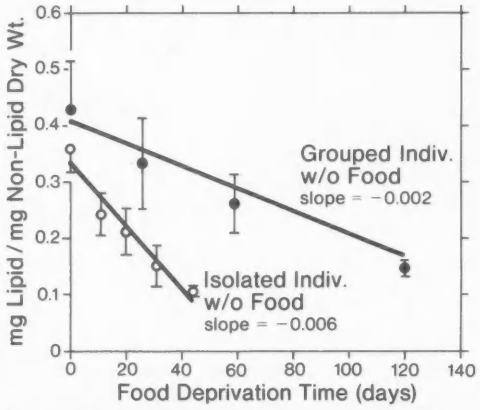

FIG. 5. Comparison of decreases in lipid content, relative to nonlipid dry weight, in grouped and individual *Pontoporeia hoyi* with time after removal of food. The grouped animals were sampled in April 1986 whereas the individual animals were sampled in December 1986. Range bars are SE.

Weight specific phosphorus release rates for the field animals peaked at about 0.12 nmole PO_4 (milligrams dry weight)$^{-1}\cdot h^{-1}$ in early July, a period when lipid content of the same animals was already decreasing (Fig. 4). Ammonium release rates also appeared to peak at this time (Fig. 2) but the peak was not as pronounced as that for phosphorus. Although the magnitude of phosphorus release appeared to be low, phosphorus release-rate patterns for the control animals were similar to those of the field animals except for a possible second peak on day 191 (Fig. 3). In contrast, the starved animals showed a minimum in phosphorus release rates (not significantly different from zero) at the end of May but then the rate progressively increased up to ca 0.2 nmole PO_4 (milligrams dry weight)$^{-1}\cdot h^{-1}$ by the middle of November (Fig. 3).

Lipid Content

In agreement with previous *P. hoyi* results in southeastern Lake Michigan (Gardner et al. 1985b; Landrum 1988), the percent lipid content of field-collected animals increased sharply between April 29 and May 25 and then decreased gradually through the rest of the year (Fig. 4). This trend was not explained by animal size, as the lipid contents was not significantly correlated with animal dry weight ($r = 0.0361$ ($n = 30$) for field-collected animals). In contrast to the seasonal trends observed for field animals, the weight-specific lipid content of the starved animals decreased to about 12% of dry weight through the first 120 d of the experiment, but then they appeared to increase during the last interval. The control animals showed trends in lipid content intermediate between those of field and starved animals. They did not exhibit the high lipid increases observed for the field animals, but showed lower rates of lipid losses than did the starved animals (Fig. 4).

Discussion

The relatively low ammonium and phosphorus excretion rates for *Pontoporeia hoyi*, that we observed at approximately in situ temperatures, could suggest a comparatively low metabolic rate that may help explain *P. hoyi*'s ability to survive long periods without food. The similarity in ammonium excretion rates between field and starved animals, and the small degree of change in rates at various intervals after food removal (Fig. 2) suggest that ammonium excretion in these benthic animals may be primarily endogenous and not substantially affected by the quality and/or quantity of food supply. The expected increase in food ingestion following the spring diatom bloom did not appear to cause our measured ammonium excretion rates to increase greatly. Ingested organic nitrogen may have been primarily assimilated into biomass during this "rapid growth" period (Johnson and Brinkhurst 1971; Johnson 1988). It is also possible that a portion of the mineralized nitrogen and phosphorus may not have been released from *P. hoyi* by direct excretion. If the mineralized nutrients were incorporated into fecal material, they would not have been measured as soluble compounds. *Pontoporeia hoyi* fecal pellets are enclosed tightly in a peritrophic membrane that is resistant to abrasion or chemical breakdown (M. Quigley, unpubl. data). The low magnitude of measured rates may also have been caused partially by the delay (up to 6 d) between sampling and the time of excretion-rate measurements. Although the animals were held in their native sediments, they may have stripped out fresh food inputs (e.g. settling diatoms) quite rapidly after samples were removed from the lake.

Alternatively, excretion rates may have been low because the animals obtained their energy from nutrients or body constituents that were low in nitrogen and phosphorus relative to carbon (e.g. carbohydrates or storage-lipids; Mayzaud and Conover 1988; Quigley et al. 1989). The decrease in lipid content relative to dry weight in the various groups of animals likely reflects the loss of triglycerides, as this lipid class is the primary energy-storage lipid in *P. hoyi* (Gardner 1985b). Comparing the ratio of respiration to ammonium excretion by *P. hoyi* (i.e. O:N ratios; Mayzaud and Conover 1988) also suggests that this animal uses more lipid than protein or carbohydrate for metabolic processes (Quigley et al. 1989).

"Weight-specific" decreases in lipid content may not reflect the full effects of starvation on the animal because simultaneous losses of other tissue components may be proportional to that of total lipids, especially after nonessential lipids are depleted. The "weight-specific lipid content" therefore may not change as dramatically during starvation as would absolute lipid content or "total weight loss" of the animals. Likewise, in the data presented above, excretion rates are based on dry weights of

the animals and do not accurately reflect changes in "absolute excretion rates" per animal during starvation. We could not obtain initial dry weights or lipid contents on the same animals that were examined during the experiment, but we were able to normalize the lipid data on starved animals to some extent by examining the "early" changes relative to lipid-free dry weight rather than to total dry weight (e.g. Fig. 5). The apparent increase in weight-specific lipid content observed after 191 d of starvation (Fig. 4) may actually reflect selective catabolism of nonlipid materials over that of essential lipids under conditions of severe starvation. The progressive increase in phosphorus release rates in the starved animals after the minimum at about 30 d may have been due in part to the catabolism of phospholipids (for energy) as triglycerides became depleted.

As indicated by the long starvation period (Fig. 2–4), grouped *P. hoyi* were able to survive in the laboratory for several months without an added food supply. Animals remaining in the starved group after the last measurements lived for up to 217 d after the experiment began. The ability of *P. hoyi* to survive this extended period in the absence of added food can likely be attributed both to the animal's low-temperature habitat and to their ability to store energy as lipids, particularly as energy-storing triglycerides. However, the "group-starved" *P. hoyi* were not necessarily completely deprived of food, as surviving animals likely obtained some nutrition from the residues of other animals that died during the experiment. This possibility was indicated by a comparison of lipid depletion rates in the grouped animals (2 μg lipid (milligrams nonlipid dry weight)$^{-1}$·d^{-1}) to those of animals starved individually for 40 d as a part of another experiment (6 μg lipid (milligrams nonlipid dry weight)$^{-1}$·d^{-1}) (Fig. 5). We made a rough estimate of the potential survival time of the latter animals based on their total available lipid and on their observed lipid-depletion rate. Assuming that storage lipids would no longer be available to the animals for energy after total lipids were decreased to about 5% of nonlipid dry weight, this calculation suggests that available lipid reserves would be adequate to support *P. hoyi* for about 50 d in the total absence of food. In addition, the animals may also obtain energy during starvation from other biochemical classes in their tissues such as proteins, as has been observed for some zooplankton (Mayzaud 1976).

The ability to accumulate lipids and live for extended periods without food may provide *P. hoyi* an advantage for survival in an ecosystem with variable food inputs (Griffiths 1977) such as the hypolimnion of Lake Michigan (Scavia and Fahnenstiel 1987; Gardner 1989a). Our data agree with the hypothesis that this organism is physiologically equipped to survive in oligotrophic, temperate lakes with seasonal inputs of diatoms. In conjunction with the spring diatom bloom, *P. hoyi* appears to grow rapidly (Johnson and Brinkhurst 1971; Johnson 1988) and accumulates energy in the form of lipids (Fig. 4), yet, it exhibits only low rates of nutrient release (Fig. 2 and 3). These characteristics reflect *P. hoyi*'s role as an efficient energy-transfer link between phytoplankton and small fish in temperate lakes where adequate silica levels support springtime blooms of large diatoms (Gardner 1989b).

Like *P. hoyi*, *Mysis relicta* and chironomids (Gardner et al. 1985b) as well as some hypolimnetic zooplankton (H. Vanderploeg and coworkers, Great Lakes Environmental Research Laboratory, Ann Arbor, Michigan, unpubl. data) in Lake Michigan have high and seasonally-variable lipid levels that may in part reflect their strategies for survival in a low-food environment. On the other hand, *Stylodrilus heringianus*, an oligochaete that lives in close proximity to *P. hoyi*, does not exhibit high or variable lipid levels (Gardner et al. 1985b). A feasible explanation for this inconsistency is that *S. heringianus* obtains its nutrition from microflora associated with particles below the sediment surface (McCall and Tevesz 1982). This feeding strategy may provide the oligochaete with a food supply that is more stable over the season than the relatively high-quality food, in the form of spring diatoms, that is seasonally available to *P. hoyi*.

Acknowledgements

We thank W. Fennel and T. Nalepa for suggestions during this study, W. Burns, J. Grimes, and D. Morse for help in collecting samples, E. Duncan, J. Malczyk, T. Miller, and H. Soo for technical advice, L. Herche for statistical assistance, and J. Chandler, T. Nalepa, and H. Vanderploeg for reviewing the manuscript.

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Determination of Picogram Levels of Methylmercury by Aqueous Phase Ethylation, Followed by Cryogenic Gas Chromatography with Cold Vapour Atomic Fluorescence Detection

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Bloom, N. 1989. Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. Can. J. Fish. Aquat. Sci. 46: 1131–1140.

A technique is presented, which allows the rapid and precise determination of methylmercury in aqueous samples. The sample is first reacted with sodium tetraethylborate, to convert the nonvolatile monomethyl mercury to gaseous methylethylmercury. The volatile adduct is then purged from solution, and recollected on a graphitic carbon column at room temperature. The methylethylmercury is then thermally desorbed from the column, and analyzed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. The method allows the simultaneous determination of labile Hg(II) species, through the formation of diethylmercury, and of dimethylmercury, which is not ethylated. The methylmercury detection limit is about 0.6 pg Hg, or 0.003 $ng \cdot L^{-1}$ for a 200-mL sample. The technique has been successfully applied directly to a wide variety of freshwater samples and alkaline tissue digestates. Seawater is analyzed following a simple extraction step to separate the methylmercury from the interfering chloride matrix. Analyses of natural surface waters have shown methylmercury levels typically in the range of 0.02–0.10 $ng \cdot L^{-1}$, with values as high as 0.64 $ng \cdot L^{-1}$ in a polluted urban lake. Waters collected from the anoxic bottom waters of a stratified remote lake have shown methylmercury levels as high as 4 $ng \cdot L^{-1}$ as Hg.

La technique présentée permet le dosage rapide et précis du méthylmercure dans l'eau. On fait d'abord réagir l'échantillon avec du tétraéthylborate de sodium, afin de convertir le monométhylmercure non volatile en méthyléthylmercure gazeux. La solution est ensuite purgée du produit volatile, qui est récupéré à température normale sur colonne au graphite. Le méthyléthylmercure est ensuite désorbé par chauffage, puis analysé par chromatographie cryogénique en phase gazeuse avec fluorodétection des vapeurs froides. La méthode permet de doser simultanément les espèces labile de Hg(II) par la formation de méthylmercure, et du diméthylmercure, qui n'est pas éthylé. La limite de détection du méthylmercure se situe à 0,6 pg de Hg, ou 0,003 $ng \cdot L^{-1}$ pour un échantillon de 200 mL. On a appliqué cette technique avec succès à une grande variété d'échantillons d'eau et de digestats de tissus alkalins. L'eau de mer fait d'abord l'objet d'une extraction simple du méthylmercure de la matrice de chlore. L'analyse des eaux de surface révèle généralement des concentrations de mercure de l'ordre de 0,02 à 0,10 $ng \cdot L^{-1}$, et des extrêmes de 0,64 ng/L dans un lac urbain pollué. Les eaux de fond anoxiques d'un lac stratifié éloigné contenaient jusqu'à 4 $ng \cdot L^{-1}$ de Hg.

Received October 4, 1988
Accepted March 9, 1989
(J9899)

Reçu le 4 octobre 1988
Accepté le 9 mars 1989

Although many lines of evidence support the conclusion that methylmercury is cycling through the biosphere, and may be disproportionately important in the overall global mercury cycle (Ballantine and Zoller 1984; Craig and Morton 1983; Jernelov 1970; Topping and Davies 1981; Westoo 1967) no adequate method has yet been introduced for the rapid, precise, and accurate determination of this species in ambient aqueous media. Solvent extraction, followed by chromatography has long been applied to the determination of monomethylmercury in tissues and sediments, where the observed levels are in the parts-per-million range (Craig and Morton 1983; Westoo 1967; O'reilly 1982; Holak 1982). Attempts to directly apply these technologies to the analysis of very large water samples have proved to be cumbersome, and to provide barely adequate detection limits for the accurate study of the environmental methylmercury cycle. These methods employ a variety of means of extraction from a large volume of water, such as resins (Robertson et al. 1987), solvents (Chiba et al. 1983; Robertson et al. 1987; Paudyn and Van Loon 1986), or sulfurated adsorbents (Lee 1987). When extracted onto a solid support, the methylmercury is then eluted and back-extracted into a solvent, prior to analysis by gas chromatography. Although each differs in detail, these methods all suffer from common drawbacks: (1) large samples are required, leading to cumbersome procedures and long processing time, (2) the overall extraction yield of the process is often uncomfortably low (50–80%), and (3) dimethylmercury, if it is present, is not detected as a separate species thus confusing our understanding of the methylmercury cycle.

In this paper, a novel method for the determination of methylmerury in water is presented, which successfully addresses the above drawbacks, while simultaneously providing a detection limit of 0.003 $ng \cdot L^{-1}$ for a 200-mL sample. This is sufficient to accurately measure methylmercury in most ambient

waters, including precipitation and remote lakes. The method reported applies an aqueous phase reaction which converts ionic mercury species to their volatile ethyl analogs (Rapsomanikis et al. 1985). In this process, aqueous tetraethylborate anion reacts with labile HgX_2 species to form diethylmercury, and with labile CH_3HgX species to form methylethylmercury. Elemental mercury and dimethylmercury, being nonionic, do not react with the tetraethylborate anion, and so also may be specifically determined. The volatile species are purged from solution onto a graphitic carbon trap, and then analyzed using cryogenic gas chromatography with a highly sensitive cold vapour atomic fluorescence detector (Bloom and Fitzgerald 1988).

Experimental Materials

Cold Vapour Atomic Fluorescence Detector

The CVAFS (cold vapour atomic fluorescence detector), described in detail by Bloom and Fitzgerald (1988), is built from readily available components. The excitation source is a 4-W low-pressure mercury vapour lamp, emitting predominantly at 254 nm (Ultra-Violet products, UVG-11), and the detector is a UV-Visible general purpose photomultiplier (Oriel 77340) shielded from stray light with a 253.7-nm interference filter (Oriel 56400). Atomic fluorescence is monitored at 90° to the direction of the excitation beam, through a 10-mm square quartz fluorescence cell (NSG Precision Cells 501F).

Cryogenic Gas Chromatograph

The cryogenic GC (gas chromatograph) column is configured as a U-tube within a sealed glass sheath (Bloom and Fitzgerald 1988), which serves to moderate the rate of heat transfer when the column is warmed during elution. The column, 80 cm in total length, is constructed from 6.4 mm outside diameter × 4 mm inside diameter borosilicate glass chromatography tubing. The sheath, also of borosilicate glass, is 2.5 cm in diameter, and 30 cm long. Prior to packing, the completed column is acid-cleaned and then silanized to deactivate the interior surfaces. The column is packed with 45 cm of preconditioned 15% OV-3 on Chromasorb W-AW-DMCS, 60/80 mesh (Bloom and Fitzgerald 1988; Andraea et al. 1981), held in place with silanized glass-wool plugs. Organomercury species are collected on the column held at −196°C in liquid nitrogen, under a flow of high purity helium. For analysis, the column is placed in a cylindrical oven held at 180°C, and the outflowing (40 $mL \cdot min^{-1}$) carrier gas passed sequentially through a 900°C thermal decomposition tube, and the CVAFS detector cell. The decomposition tube consists of an 8-inch length of 9.5 mm outside diameter × 6 mm inside diameter quartz tubing with the central 7.6 cm packed with quartz wool. The tube is electrically heated with a winding of 18 ga nichrome resistance wire. Thermal decomposition is necessary to render all eluted species as Hg°, the only form detectable by CVAFS.

Fittings and Tubing

Connections between components and columns are made using 6.4 mm or 9.5 mm outside diameter Teflon FEP tubing, and Teflon friction-fit or threaded tubing connectors. Friction-fit sleeves are 3-cm sections cut from unshrunken 6.4 mm or 9.5 mm inside diameter Teflon TFE heat-shrinkable tubing (Cole-Parmer Inst. Co.). Connections between components requiring mobility are made with 3.2 mm outside diameter Teflon FEP tubing, because of its greater degree of flexibility. All Teflon components are cleaned in hot concentrated HNO_3 prior to use.

Purge Vessel

The purge vessel is constructed from a 250-mL standard taper 24/40 glass-stoppered Erlenmeyer flask, with a special 4-way valve sparging-tube cap-assembly (Laboratory Data Control 700542). This valve assembly allows the water sample to react initially with the ethylating reagent, without bubbling, then to be purged onto the trapping column, and finally to be bypassed, so that water vapour adsorbed onto the column may be evaporated by the direct flow of dry carrier gas.

Carbotrap Columns

Columns for the pretrapping of purged organomercury species are constructed from 6.4 mm outside diameter × 4.0 inside diameter silanized borosilicate chromatography tubing. The columns are 15.2 cm long, and packed with 7.6 cm of 20/40 mesh Carbotrap (graphitized carbon black, Supelco 2-0827), held in place with silanized glass wool plugs (Bloom and Fitzgerald 1988).

Gases

Helium is laboratory grade (Airco), further purified to <1 ppm O_2 with a high-temperature gas purifier (Supelco 2-3802). The gas is then passed through a gold-coated sand trap (Fitzgerald and Gill 1979) to remove mercury prior to use. Nitrogen and air are laboratory grade, passed first through activated carbon, then through gold-coated sand prior to use.

Water

All rinsing of containers and aqueous dilutions are made with tap water that has been running for at least 1 h before use. This water is found to be extremely low, and constant, in total mercury (0.3 $ng \cdot L^{-1}$), at least one order of magnitude lower than our best laboratory deionized water. No methylmercury has been detected in our laboratory tap water, which comes from an on-site deep well.

Gas Phase Mercury Standards

Mercury standards are taken by airtight gas syringe from the headspace over the volatile compound of interest (Bloom and Fitzgerald 1988; Dumarey et al. 1985). For Hg°, about 5 g of the pure liquid is placed in a 250-mL amber glass bottle fitted with a septum-cap. For dimethylmercury, a 14 $mg \cdot L^{-1}$ solution is prepared in octanol, and 25 mL placed into the bottle. A 200 $mg \cdot L^{-1}$ solution is used for diethylmercury, owing to its lower vapour pressure. The organomercurial standards are calibrated daily against Hg°, using the gold-column/CVAFS system (Fitzgerald and Gill 1979).

Methylmercury Standard

Excess of methylmercuric chloride (Alfa 37123) is equilibrated with GC grade isopropanol at 20°C, to form a saturated solution. A concentrated stock solution, prepared by 1:1000 v/v dilution of the above in isopropanol, has been found to contain 4.16 $mg \cdot L^{-1}$ total mercury, and 4.02 $mg \cdot L^{-1}$ nonvolatile organic mercury, all of which is assumed to be in the mon-

omethyl form. A working standard solution prepared by a nominal 1:800 v/v dilution of the above in isopropanol, has repeatedly been analyzed and found to contain 5.40 $\mu g \cdot L^{-1}$ Hg as CH_3HgCl. This solution has been found to maintain both its total mercury concentration, as well as its methylmercury concentration for over 12 mo when stored in an amber glass bottle with Teflon cap, at a temperature of 0–5°C.

Sodium Tetraethylborate Solution

An approximately 1% solution of sodium tetraethylborate (STREM 11-0575) is freshly prepared every 2–3 d. Since this reagent is extremely air sensitive, it is not weighed, but rather measured by volume. Approximately 0.5 g (c.a. 2 cm^3) of the solid are quickly removed from the bottle, and added to 50 mL of cold water in a small Teflon FEP bottle. This solution is stored in the refrigerator except during use.

Sodium Acetate Buffer

Two moles of reagent grade sodium acetate (272 g) and 2 mol of Ultrex glacial acetic acid (118 mL) are dissolved in tap water to give a final volume of 1 L. This solution is purified of trace mercury by the addition of 5 g of 1N HCl-rinsed sulfhydryl chelating resin (Sumitomo Q-10R) to the bottle, and periodic agitation over a span of several days. The solution is then filtered, and stored in a Teflon FEP bottle, at room temperature.

Potassium Hydroxide/Methanol

Reagent grade KOH pellets (250 g) are dissolved in high purity methanol, to a final volume of 1 L. The solution is stored in a Teflon FEP bottle at room temperature.

Other Reagents

All acids, bases, and sodium chloride are reagent grade. Methylene chloride, methanol, and isopropanol are all high purity GC grade. All are stored in acid-cleaned Teflon FEP bottles, or glass bottles with Teflon FEP caps.

Procedures

Methylmercury in Freshwater Samples

Illustrated in Fig. 1 is a schematic diagram of the major steps involved in the procedure outlined below. A 100- to 200-mL water sample is placed into a 250-mL purging flask. Acetate buffer (200 μL) is added, bringing the pH to 4.9 ± 0.1. Sodium tetraethylborate solution (50 μL) is added, the flask swirled to mix, and the 4-way valve-cap inserted. The mixture is allowed to react without purging for 20 min, and then is purged with N_2 or air at a flowrate of 250 $mL \cdot min^{-1}$ for 25 min. The purge gas outflow is passed through a Carbotrap column to collect the volatile organomercury compounds. After the sample is purged,

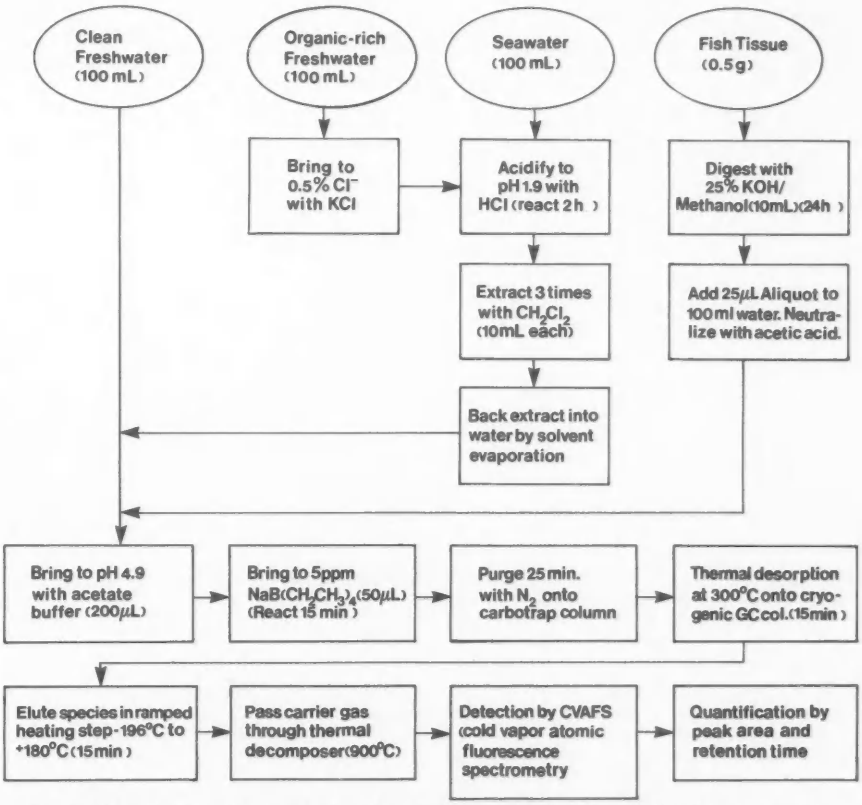
Clean Freshwater (100 mL)
Organic-rich Freshwater (100 mL)
Seawater (100 mL)
Fish Tissue (0.5 g)
Bring to 0.5% Cl⁻ with KCl
Acidify to pH 1.9 with HCl (react 2 h)
Digest with 25% KOH/ Methanol (10 mL) (24 h)
Extract 3 times with CH_2Cl_2 (10 mL each)
Add 25 μL Aliquot to 100 ml water. Neutralize with acetic acid.
Back extract into water by solvent evaporation
Bring to pH 4.9 with acetate buffer (200 μL)
Bring to 5ppm $NaB(CH_2CH_3)_4$ (50 μL) (React 15 min)
Purge 25 min. with N_2 onto carbotrap column
Thermal desorption at 300°C onto cryogenic GC col. (15 min)
Elute species in ramped heating step -196°C to +180°C (15 min)
Pass carrier gas through thermal decomposer (900°C)
Detection by CVAFS (cold vapor atomic fluorescence spectrometry
Quantification by peak area and retention time

FIG. 1. Schematic flow diagram showing the principal steps in the determination of mercury species by aqueous phase ethylation and cryogenic GC separation. See text for a detailed description of each step.

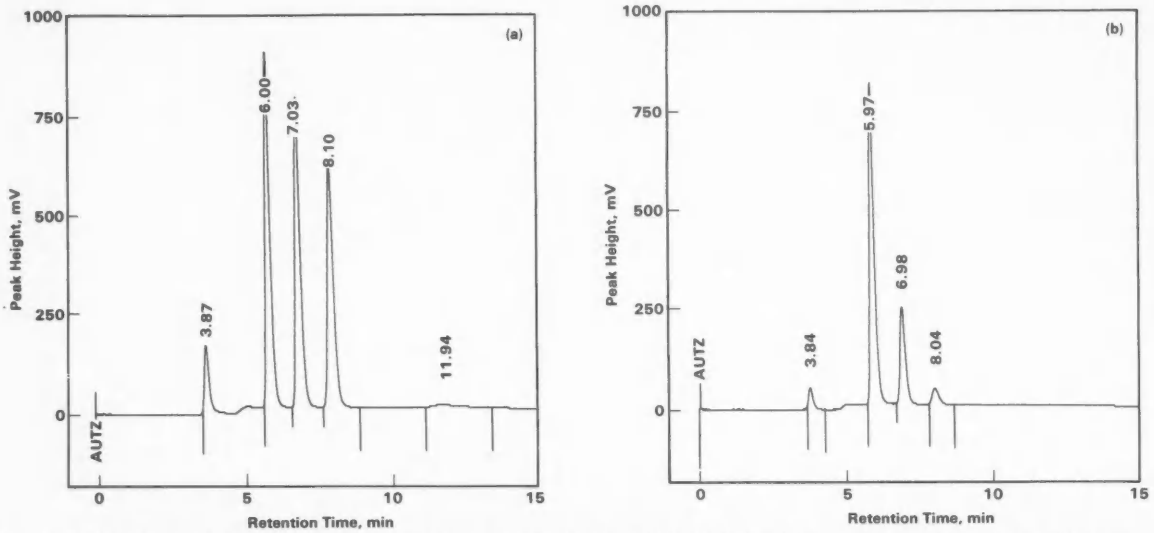

FIG. 2. Typical chromatograms of organomercury species obtained by aqueous phase ethylation. (a) tap-water sample containing spikes of 454 pg Hg as $(CH_3)_2Hg$ (6.00 min peak), 429 pg Hg as CH_3HgCl (7.03 min peak; methylethylmercury), and 400 pg Hg as $HgCl_2$ (8.10 min peak; diethylmercury). Peak at 3.87 min is residual Hg°. (b) Fish tissue digestate, equivalent to about 1 mg *Catostomus commersoni*, spiked with 413 pg Hg as $(CH_3)_2Hg$) dimethylmercury.

the valve is switched, to pass dry gas over the column for 7 min, to remove residual water condensation from the trap.

The Carbotrap column is then connected in-line with the GC column, which is held in liquid nitrogen. The Carbotrap column is placed so that the end facing the bubbler output is now toward the GC column input. This avoids passing the organomercurial species down the entire length of the heated Carbotrap column during desorption, thereby greatly minimizing on-column breakdown of diethylmercury to the monoethyl form (Bloom and Fitzgerald 1988). Helium is allowed to flow through the cool Carbotrap column for 3 min, at 90 $mL \cdot min^{-1}$, and then the column is electrically heated with 22 ga nichrome resistance wire for 10 min at a temperature of 300°C, under continuous helium flow.

Once sample desorption is completed, the cooled Carbotrap column is removed from the system, and the thermal decomposition tube and recorder/integrator are turned on. The GC column is then removed from the liquid nitrogen, and placed into a cyclindrical oven held at 180°C. The mercury species are eluted in order of increasing molecular weight with the following retention times Hg°, 4.17 ± 0.07 min; $(CH_3)_2Hg$, 6.23 ± 0.14 min; $CH_3CH_2HgCH_3$, 7.25 ± 0.09 min; and $(CH_3CH_2)_2$ Hg, 8.38 ± 0.17 min (Fig. 2). Elemental mercury is present both as a residual from the heating of the column, and as a potential breakdown product of the organomercurials, and hence should be minimal. Hg° present in the aqueous sample passes through the Carbotrap column during the purging/drying steps and may be collected on a back-up gold trap. Methylethylmercury is the ethyl derivative of monomethylmercury, and diethylmercury is the ethyl derivative of inorganic labile mercuric species. Dimethylmercury is purged and detected unchanged by this method.

Small samples, such as tissue digests, pore water, etc., may be analyzed by dilution with tap water, and analysis as above. A smaller (25 mL) bubbler is used in these cases, and the purge time reduced to 10 min.

Preextraction of Samples Containing Complexing Agents

Because high levels of chloride (>200 ppm) are observed to inhibit the ethylation process, methylmercury in seawater must first be extracted from the chloride-rich matrix, and then be returned to a freshwater matrix. This is done by extracting a 100-mL sample, acidified to a pH of 2–5, with 3 consecutive 10-mL portions of methylene chloride, using a Teflon FEP separatory funnel. The methylene chloride extracts are combined in a 125-mL Teflon bottle, and the surface rinsed with 5 mL of tap water, without shaking, to remove residual saltwater droplets on the solvent surface. The tap water rinse is removed by pipetting. About 70 mL of tap water is then added to the methylene chloride, and the bottle placed in a hot water bath at 60°C until all of the CH_2Cl_2 has boiled away. The water is then purged for 10 min at 250 $mL \cdot min^{-1}$ with N_2 to remove residual solvent. The monomethylmercury is now transferred to the freshwater matrix, which is analyzed as before.

This procedure may also be used to extract the total methylmercury component from freshwater samples containing organic complexing agents, high transition metals concentrations, or particulate matter which inhibits the complete recovery of methylmercury by the direct ethylation procedure. In these cases, 5 mL of a solution containing 30% KCl and 2% HCl are added to 100 mL of the sample and allowed to equilibrate for 2–4 h. The sample is then extracted as above.

Digestion Procedure for Soft Tissues

Approximately 1.0 g of homogenized wet tissue, or 0.25 g of pulverized freeze-dried tissue is accurately weighed into a 22-mL Teflon vial (Savillex 0275), and 10.0 mL of 25% KOH/methanol solution are added. The vial is vigorously shaken and the tissue is allowed to digest at room temperature for 24–48 h, or is placed in an ultrasonic bath for 1 h. The digest is then diluted to 20.0 mL with methanol. To analyze, 50 µL of the extract are added to 100 mL of tap water, and neutralized with

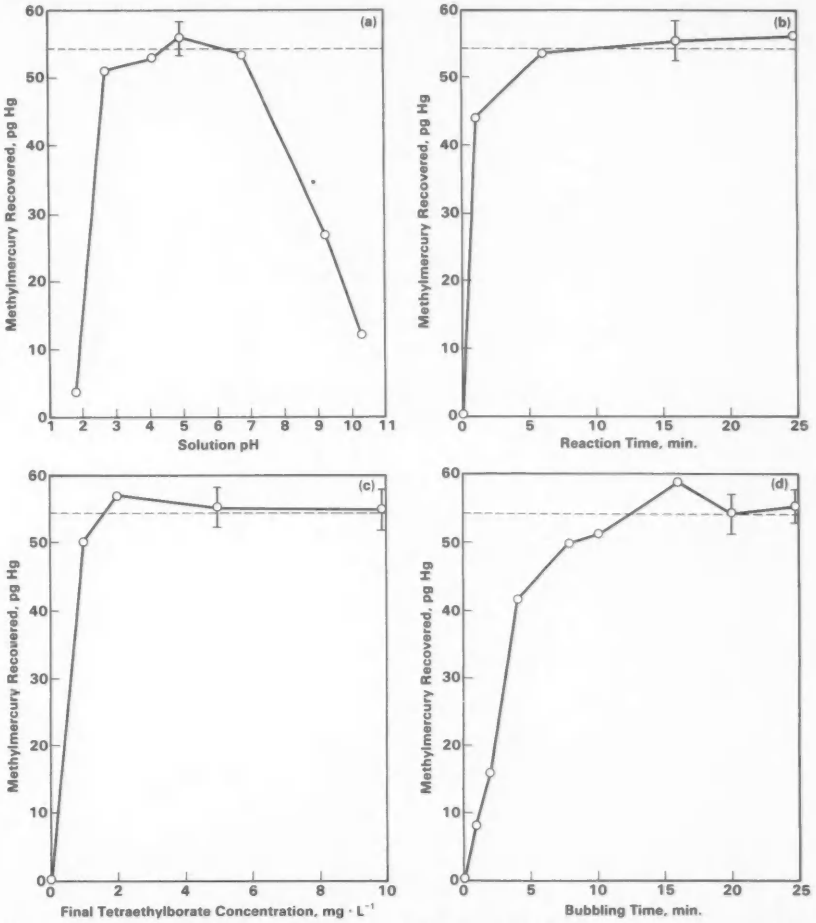

FIG. 3. Optimization of ethylation parameters. (a) Effect of pH. (b) Effect of ethylation time before bubbling. (c) Effect of sodium tetraethylborate concentrations in reaction flask. (d) Effect of bubbling time. Line at 54.7 ng recovery indicates spike addition (expected yield). Error bars represent the standard deviation of 9 to 11 replicates.

50 μL 2.0 M acetic acid. After the addition of 200 μL of acetate buffer, the sample is analyzed as for a freshwater sample.

Results and Discussion

Optimization of Ethylation Parameters

Rapsomanikis et al. (1985) in their study of lead ethylation judged the following parameters as most important to optimize: pH, purge time and flow, and amount of reagent. While these authors used simplex optimization as a guide, the present work was based upon the more traditional systematic approach, which results in a clearer understanding of the importance of each factor, once the data have been collected. After using trial and error to obtain initial parameters which gave a strong signal, one parameter was systematically varied while holding the others constant. When an optimum was found for that parameter, it was then held constant at that level while the other parameters were varied in their turn. Once all of the optima were located, the process of varying each parameter in its turn, while holding all others at their optima was repeated to generate the data illustrated in Fig. 3a–d. For all of these determinations, 100 mL of tap water, spiked to contain 0.54 $ng \cdot L^{-1}$ Hg as monomethylmercury was used as the test solution.

As can be seen in Fig. 3a, the pH of ethylation was a critical parameter, showing a broad optimum in the range of 4–7, and tailing off dramatically at both extremes. The yield diminishes at low pH because of rapid destruction of the ethylating agent by H^+, while at high pH, the loss in yield seems to be caused by inhibition of the ethylation reaction — perhaps due to the formation of unreactive multihydroxyl methylmercury anions. As a matter of convenience, a pH of 4.9 was chosen, as this may be obtained using a 1:1 acetic acid/sodium acetate buffer. Fig. 3b and 3c show that the ethylation reaction was both very rapid, and required little reagent. The amount of reagent chosen, 50 μL of a 1% solution (5 ppm concentration in the sample) was in excess of the minimum necessary, but not so high as to lead to interferences in the GC elution, as will be discussed below. A reaction time of 20 min, and purge time of 25 min

TABLE 1. Inter-day system precision for methylmercury determinations in water (100-mL samples).

Number of replicates	Expected concentration ($ng \cdot L^{-1}$)	Observed concentration ($ng \cdot L^{-1}$)	Relative standard deviation (%)
Many	0	<0.006	—
10	0.06	0.064 ± 0.005	7.8
13	0.28	0.296 ± 0.016	5.4
9	0.55	0.549 ± 0.029	5.3
10	(1.355)[a]	1.355 ± 0.053	3.9

[a]Standards, calibrated as total Hg against gas phase Hg°.

(Fig. 3d) gave quantitative results without prolonging the analysis time.

Interferences

A variety of common chemical species were added to methylmercury-spiked tap water (0.54 $ng \cdot L^{-1}$ Hg as CH_3HgCl) prior to ethylation, to determine potential interferences. Common environmental anions such as sulfate, nitrate, and phosphate were found to be noninterfering (>95% spike recovery as methylethylmercury) at levels up to at least 5000 ppm. Chloride, however, was found to strongly interfere, reducing recovery to 95% at 200 ppm, and to less than 10% at 5000 ppm. Natural freshwaters, precipitation, and tissue digestates rarely contain levels of halides found to be strongly inhibitive. For the analysis of seawater, however, the methylene chloride preextraction must be applied to separate the methylmercury from the bulk of the interfering chlorides.

A mixture of cations containing Fe(III), Co(II), Cu(II), Pb(II), Zn(II), Ni(II), and Mn(II) each at the 1.0 ppm level was found to be noninhibiting to the determination of methylmercury. At the 10 ppm level, a colloidal precipitate of Fe(III) hydroxide was observed, as well as 50% inhibition of CH_3Hg recovery. Calcium, as the nitrate, at a level of 5000 ppm was found to be noninhibiting. Since natural waters rarely contain over a few parts per billion of transition metals, cationic interferences are not expected. When analyzing waters high in these species, such as waste streams and sediment pore waters, the addition of NaCl and preextraction with CH_2Cl_2 may be warranted.

All soluble organic materials tested, including methanol, isopropanol, ethanol, acetone, methylene chloride, and acetates were found to have no inhibitory effects at levels of up to 1000 $mg \cdot L^{-1}$. A dramatic reduction in yield was observed with several organic rich surface and bottom waters collected during the summer algal bloom at Little Rock Lake (Wisconsin). In these cases typically 30–40% of the total methylmercury added as a field spike was recoverable by the direct ethylation procedure. This was thought to result from organic complexation, because repeated reethylation of the sample continued to result in additional recovery of the spike. Application of methylene chloride extraction following the addition of KCl/HCl resulted in 90–100% methylmercury recoveries for these waters.

A serious interference was noted when polluted lake water, clearly containing heavy industrial oils, was run directly through the ethylation process. In this case, the sensitivity of the OV-3 column was rapidly destroyed, necessitating the construction of a new analytical column. This problem was overcome by passing the gas stream from the bubbler through a 1.0-cm disposable trap packed with 60/80 mesh 15% OV-3 on Chromosorb W-AW DMCS. This column effectively trapped the high molecular weight oil fumes, while passing the dialkylmercury species through.

Another destructive interference was noted with the addition of excessive sodium tetraethylborate to the bubbler. At levels greater than 50 ppm final concentration (0.5 mL of 1% solution per 100 mL of sample), the Carbotrap column was progressively damaged. The result was that the diethylmercury collected was partially decomposed to monoethylmercury during the thermal desorption step. No effect was noted with respect to the methylethylmercury peak, but as a precaution, traps showing greater than 10% decomposition of the diethyl peak were discarded. Decomposition was detected by the appearance (of a broad peak at about 12.4 min) retention time, corresponding to monoethylmercury elution (Bloom and Fitzgerald 1988).

The ethylating reagent was also found to contribute a specific inhibitory interference for dimethylmercury, caused by the overlap of the $(CH_3)_2Hg$ and $(C_2H_5)_3B$ peak elution times. The approximately 80% inhibition of the dimethylmercury peak may be eliminated either by purging the bubbler with air, rather than an inert gas, or by analyzing a duplicate of each sample, with no added ethylation reagent, to obtain only the dimethylmercury component. The use of air removes the interference because oxygen reacts with the triethylborane to form a less volatile species, which is not coeluted with any of the analyte peaks. However, build up of nonvolatile boron compounds may shorten the useful life of Carbotrap columns.

Precision, Accuracy, and Detection Limits

Replicate analyses of methylmercury spiked tap water at several concentrations were conducted over a period of several weeks, thus giving an indication of the inter-day precision of the method (Table 1). Within-day relative standard deviations were approximately one-half of those reported in Table 1.

The detection limit of the system for methylmercury was a function of the sample size, as no reagent blanks were observed. The instrumental detection limit, three times the instrumental noise, was about 0.6 pg as Hg. Thus, using a 200-mL sample volume, the detection limits for both monomethylmercury and dimethylmercury were 0.003 $ng \cdot L^{-1}$ as Hg. The detection limit for labile Hg(II) species was dependent upon reagent contamination, and was typically higher than the detection limits attained for the analogous "acid labile mercury" using $SnCl_2$ reduction and trapping on gold (Bloom and Crecelius 1983). With a detection limit of about 0.2 $ng \cdot L^{-1}$ (3 σ of the reagent blank), however, it was still feasible to use this method to collect simultaneous values for methylmercury and inorganic mercury. Work is under way to devise methods to further clean up the system, and purify the sodium tetraethylborate solution of inorganic mercury contamination.

No independent verification of the system accuracy on low-level aqueous samples was performed, due to the lack of both certified standards, and of other laboratories capable of making such measurements. However, for inorganic mercury, there exists a high-level aqueous standard, NBS-1641b, which was analyzed both by traditional $SnCl_2$ reduction, and as the diethyl peak using the methods described here (Table 2). For monomethylmercury, digestions of NBS-50 albacore tuna (*Thunnus alalunga*) were used, showing the determination of both total Hg, by hot acid digestion and $SnCl_2$ reduction, and methylmercury by the methods of this paper. Dimethylmercury recovery could only be assessed by spiking samples with a solution

TABLE 2. Analysis of standard materials for mercury speciation.

Material	Observed mercury concentrations			
	Total Hg[a]	Hg (II)[b]	CH_3Hg[b]	$(CH_3)_2Hg$[b]
NBS-16416	1.40	1.24	<0.003	<0.003
Aqueous Hg(II)	1.34	1.46	<0.003	<0.003
($\mu g \cdot mL^{-1}$)	1.42	1.20	<0.003	<0.003
$\bar{X}$	1.39 ± 0.04	1.30 ± 0.14	<0.003	<0.003
Certified[c]	1.52 ± 0.02	—	—	—
NBS-50	0.913	<0.04	0.896	<0.005
Albacore tuna	0.801	<0.04	0.803	<0.005
($\mu g \cdot g^{-1}$ dry wt.)	0.846	<0.04	0.853	<0.005
	0.870	—	—	—
$\bar{X}$	0.858 ± 0.047	<0.04	0.851 ± 0.047	<0.005
Expected[d]	0.95 ± 0.10	—	(0.76–0.86)	—

[a]BrCl oxidation (Bloom and Crecelius 1983).
[b]Ethylation procedure.
[c]Long experience at this laboratory, using three different techniques, three analysts, and six instruments yields a value of 1.38 ± 0.12 $\mu g \cdot mL^{-1}$.
[d]This standard research material has never been certified.

TABLE 3. Extraction efficiency of CH_3HgCl from 3.0% NaCl solution as a function of pH and number of methylene chloride extractions. Each sample contained a spike of 152 pg CH_3HgCl in 100 mL of solution.

Sample pH	Number of 10-mL CH_2Cl_2 extractions			$K = ([CH_3Hg]Solv/[CH_3Hg]_{aq})$
	1	2	3	
1.9	105 ± 4 pg (68.8 ± 2.5%)	140 ± 8 pg (92.1 ± 5.4%)	152 ± 5 pg (100.1 ± 3.5%)	22.1 ± 0.8
4.9	102 ± 3 pg (67.1 ± 2.0%)	136 ± 19 pg (89.4 ± 11.1%)	152 ± 6 pg (100.0 ± 6.1%)	20.3 ± 0.6
7.5	29 ± 1 pg (19.1 ± 0.8%)	51 ± 4 pg (33.8 ± 2.6%)	75 ± 8 pg (49.6 ± 2.0%)	1.4 ± 0.1

TABLE 4. Extraction of unfiltered acidified seawater with 2 × 10 mL CH_2Cl_2, followed by analysis for methylmercury by ethylation and cryogenic GC/CVAFS. Spiked samples contained 0.304 $ng \cdot L^{-1}$ CH_3HgCl added.

Sample	Replicate extractions, $ng \cdot L^{-1}$ CH_3HgCl				
	No. 1	No. 2	No. 3	$\bar{X}$	σ
100 mL Sequim Bay water (SBW)	0.061	0.054	0.061	0.059	0.004
Blank (reextract of above)	0.039	0.044	0.042	0.041	0.003
Net (SBW) (minus mean blank)	0.019	0.012	0.019	0.017	0.004
100 mL SBW plus 30.4 pg as Hg CH_3HgCl	0.340	0.312	0.371	0.341	0.030
Net spike recovery	0.281	0.253	0.312	0.282	0.030

of $(CH_3)_2Hg$, which was previously analyzed for total Hg. This is discussed below under the heading fish tissues.

Storage Experiments

Short-term storage experiments were conducted at methylmercury levels of 0.057 $ng \cdot L^{-1}$ Hg and 0.128 $ng \cdot L^{-1}$ Hg for several storage conditions. The 100-mL aliquots of spiked tap water were stored in identically treated sets of 12 125-mL acid-cleaned Teflon bottles, and individual bottles randomly selected for analysis over a period of 1 mo. Two bottles were analyzed at each time interval. These experiments showed all aliquots to be within ± 10% of the initial concentration over the entire month for the following two conditions: (1) quick-freezing in liquid nitrogen, followed by storage at −80°C (Crecelius et al. 1986), and, (2) storage at 0–4°C, in the dark. Samples stored at room temperature, under fluorescent lighting, showed an approximately 20% decrease in the CH_3HgCl over 1 mo, although no change was evident in the first 2 wk. Samples stored at room temperature after acidification with 0.1% HCl showed a rapid loss of methylmercury, with >80% being lost by the second day. Until further more comprehensive long-term studies are conducted, it is recommended that unacidified samples be quick-frozen for long-term storage.

Extraction of Methylmercury from Seawater

The extraction of efficiency of monomethylmercury from a 3% sodium chloride solution was examined, as a function of pH, using methylene chloride as the organic phase (Table 3). In all determinations, 100 mL of solution, spiked to contain 1.52 $ng \cdot L^{-1}$ Hg as CH_3HgCl, was extracted with 10-mL ali-

TABLE 5. Recovery of mercury species spiked into 1.0-g aliquots of freshwater white sucker (*Catostomus commersoni*) tissue prior to KOH/methanol digestion and analysis by ethylation/GC.

	Unspiked tissue (as Hg, ng·g^{-1}, wet wt.)			Spiked tissue, net values (as Hg, ng·g^{-1}, wet wt.)		
Replicate	CH_3Hg	Hg(II)	$(CH_3)_2Hg$	CH_3Hg	Hg(II)	$(CH_3)_2Hg$
1	250	<4.5	<2.2	359	205	303
2	257	<4.5	<2.2	495	247	301
3	241	<4.5	<2.2	371	209	321
4	232	<4.5	<2.2	453	—	—
5	216	<4.5	<2.2	449	—	—
6	245	<4.5	<2.4	336	—	—
$\bar{X}$	240	<4.5	<2.4	410	220	308
σ	14	—	—	64	23	11
Spike (ng)	—	—	—	401	260	316
% Recovery	—	—	—	102.4	84.6	97.5

TABLE 6. Methylmercury and total mercury contents of various freshwater and marine fish.

Fish species	Replicates	Mercury concentrations (μg·g^{-1} wet wt.) Methylmercury	Total mercury	Methylmercury (%)
Chinook salmon (*Oncorhynchus tshawytscha*)	5	0.0418 ± 0.0042	0.0430 ± 0.0031	97.2
Pacific halibut (*Hippoglossus stenolepis*)	4	0.0555 ± 0.0080	0.0653 ± 0.0018	85.0
Yellow perch (*Perca flavescens*)	3	0.526 ± 0.070	0.568 ± 0.026	92.3
White sucker (*Catostomus commersoni*)	3	0.249 ± 0.008	0.231 ± 0.015	107.8
Northern pike (*Esox lucius*)	3	1.762 ± 0.072	1.661 ± 0.043	106.1

TABLE 7. Observations of mercury speciation in unfiltered natural waters collected in 1988. No dimethylmercury was observed in any sample.

			Mercury concentrations (ng·L^{-1} as Hg)			
Water type	Location	Date	Total	Labile	Monomethyl	*N*
Oligotrophic (surface)	Lake Crescent, WA	3/16/88	0.163	0.170	<0.004	3
Mesotrophic (surface)	Lake Union, WA	2/25/88	1.69	0.072	0.039	4
Eutrophic (surface)	Onadaga, NY	2/28/88	10.68	0.523	0.636	2
Seawater (surface)	Sequim Bay, WA	5/2/88	0.357	0.089	0.016	3
Artesian well	Sequim, WA	6/29/88	0.289	0.183	<0.002	2
Stratified (surface)	Little Rock, WI	9/10/88	1.06	0.03	0.125[a]	2
Stratified (bottom)	Little Rock, WI	9/10/88	10.58	1.38	3.96[a]	2

[a]These values were obtained following CH_2Cl_2 extraction from samples treated with KCl/HCl. Values obtained by direct ethylation of the organic-rich waters were 0.041 ng·L^{-1} Hg (surface) and 1.21 ng·L^{-1} Hg (depth).

quots of methylene chloride. Results are the mean and standard deviation of triplicate analyses. Using the data for the single extractions of the samples, the distribution coefficient, $K = [CH_3HgCl]_{solv}/[CH_3HgCl]_{aq}$ was calculated. These values are important for determining the amount of solvent (V_{solv}) needed to effectively extract other volumes of sample (V_{aq}), which may be determined by calculating the extraction efficiency (E):

$$E = \frac{K}{K + (V_{aq}/V_{solv})}.$$

The distribution coefficient was observed to decrease with increasing pH, from 22.1 at pH = 1.9 to only 2.4 at a pH of 7.4. This is in accord with the observations of others (Robertson et al. 1987), and makes it clear that a sample containing methylmercuric chloride must be acidic to allow quantitative extraction into an organic solvent. It is also clear that strong acidification is not necessary for good yield, the distribution coefficients in this case being almost identical between pH 2 and pH 5. Since dimethylmercury is converted to monomethylmercury in the extraction procedure, untreated samples must be separately purged onto Carbotrap for the determination of $(CH_3)_2Hg$ in seawater.

For the extraction of seawater, the working conditions were chosen to be acidification to pH = 1.9 (1.0 mL 12.7 N HCl

per litre of sample), followed by three consecutive 10-mL extractions with CH_2Cl_2. Table 4 illustrates the results of this procedure on spiked unfiltered water taken by hand-dipping from Sequim Bay, WA. This water was found to contain 0.357 $ng \cdot L^{-1}$ total mercury, and 0.089 $ng \cdot L^{-1}$ "acid labile" mercury. The salinity was about 30‰. The blanks were determined by again subjecting the previously extracted sample to exactly the same reagents and procedures. As can be seen, there was a small reagent blank of 0.042 ± 0.003 $ng \cdot L^{-1}$ Hg methylmercury associated with the procedure. The source of this contamination was traced to the methylene chloride. The contamination could be reduced significantly by preextracting the solvent with 1.0 N NaOH in deionized water. The seawater samples were spiked with 0.32 $ng \cdot L^{-1}$ methylmercury to give a more realistic test of the procedure at low concentrations. When the small (0.016 $ng \cdot L^{-1}$) level of methylmercury already present in the water was considered, the recovery of the spike was observed to be 93.4 ± 9.4%.

Fish Tissues

Mercury speciation was analyzed from spiked and unspiked frozen samples of white sucker (*Catostomus commersoni*) collected from a small midwestern lake (Table 5). The yields for both monomethylmercury and dimethylmercury on these samples were close to 100%, while that for Hg(II) was lower, about 85%. Dimethylmercury recoveries reported were assessed by separate analysis of a nonethylated aliquot of fish tissue digestate diluted in 100 mL of tap water. Results of recoveries from the ethylation procedure, using air purging to eliminate the triethylborane inhibition were similar, although the variability was a factor of five greater.

Since each spike was added to a separate sample, an assessment of species interconversion was possible. In no case was any spike observed to result in the recovery of any species other than that which was added. The low yield for Hg(II) resulted from the partial conversion of the ionic form to Hg°, which was not retained by the Carbotrap, but was collected on a back-up gold trap. The higher variability of the monomethylmercury yield was attributable to the difficulty in pipetting the small volumes of organic-based sample extract necessary to keep the system on scale.

The digestion procedure outlined was applied to several species of fresh and saltwater fish (Table 6). In addition to mercury speciation analysis, total Hg analysis by hot concentrated HNO_3 digestion, followed by $SnCl_2$ reduction and gold trapping was employed. These results indicated that almost all of the mercury observed in these tissues was in the form of monomethylmercury, with undetectable levels of either labile inorganic or dimethylmercury. The exception was halibut (*Hipoglossus stenolepis*) which was observed to contain only 86% of its total as monomethylmercury, the rest being labile Hg(II) species.

Analysis of Environmental Waters

Naturally occurring waters from a variety of sources were analyzed to assess the adequacy of the methods and detection limits for application in the field (Table 7). Most surface waters investigated were found to contain low levels of monomethylmercury, with the highest levels (0.64 $ng \cdot L^{-1}$) being observed in the surface waters of a mercury and organic waste polluted urban lake (Onadaga Lake, Syracuse, NY). Dramatic elevations of methylmercury concentrations in the anoxic bottom waters of a stratified seepage lake (Little Rock Lake, WI) were observed through the summer of 1989, indicating a sediment source for methylmercury to the water column of this lake. Some representative data from this system are presented in Table 7, although the complete data set will be the subject of a future publication. Precipitation samples have been found to contain significant levels of monomethylmercury often approaching 5% of the total mercury content of the sample (Bloom and Watras 1989). No dimethylmercury was observed in any water sample collected for this study, although a deep, anoxic water sample collected 1 yr earlier from Onadaga Lake did show dimethylmercury at a level of 0.41 $ng \cdot L^{-1}$.

Conclusions

These results are an important positive proof of the ubiquity of methylmercury cycling in the environment, and indicate that the methods described in this paper are adequate for detailed quantitative studies of these processes. Studies are under way to assess the role of the methylmercury cycle within the overall global mercury cycle. The results should help clarify the role played by acid rain in the enhancement of methylmercury bioaccumulation by freshwater fish.

Acknowledgements

The results presented in this paper were obtained during work funded by the Electric Power Institute, under contract No. 25120-11173, and by Tetra Tech (California) under contract No. 25120-05850. Special thanks to Allyn A. Bloom for his efforts in catching many of the fish used for development of analytical methods.

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Dietary Lipid as a Factor Modulating Xenobiotic Metabolism in Channel Catfish (*Ictalurus punctatus*)

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Ankley, G. T., V. S. Blazer, S. M. Plakas, and R. E. Reinert. 1989. Dietary lipid as a factor modulating xenobiotic metabolism in channel catfish (*Ictalurus punctatus*). Can. J. Fish. Aquat. Sci. 46: 1141–1146.

Adult channel catfish (*Ictalurus punctatus*) were fed diets containing menhaden oil (MHO), soybean oil (SBO), or beef tallow (BFT) as lipid sources for 116 d. The effects of these diets on two important hepatic xenobiotic-metabolizing enzyme systems, cytochrome P-450-dependent monooxygenases (MOs) and glutathione *S*-transferases (GSTs), were evaluated. Microsomal MO and cytosolic GST activities were consistently greater in fish fed MHO than in those receiving SBO or BFT. Generally, enzyme activities in fish fed SBO and BFT were similar. The different diets also markedly influenced the fatty acid composition of the liver, particularly with respect to the relative abundance of specific fatty acid families. These data demonstrate that dietary lipid does affect the activities of xenobiotic-metabolizing enzymes in fishes, and thereby could influence the sensitivity of fishes to certain organic toxicants.

On a soumis pendant 116 jours la barbue adulte de rivière (*Ictalurus punctatus*) à des régimes alimentaires comportant soit de l'huile de menhaden, soit de l'huile de soja, soit du suif de boeuf comme sources de lipides. On a évalué les effets de ces régimes sur les glutathions-*S*-transférases et les mono-oxygénases dépendantes du cytochrome P-450, deux des principaux systèmes enzymatiques du foie, qui métabolisent les substances xénobiotiques. Les activités microsomiques et cytosoliques de la glutathion-*S*-transférase étaient régulièrement supérieures chez les poissons ayant consommé de l'huile de menhaden comparativement à ceux à qui l'on avait offert de l'huile de soja ou du suif de boeuf. De façon générale, les activités enzymatiques chez les poissons ayant ingéré ces deux derniers lipides étaient semblables. Les différents régimes alimentaires ont également eu un effet marqué sur la composition des acides gras dans le foie, surtout en ce qui a trait à l'abondance relative de certaines familles de ces substances. Ces résultats démontrent que les lipides alimentaires ont un effet réel sur les activités des enzymes qui métabolisent les substances xénobiotiques, ce qui peut modifier la vulnérabilité des poissons à certains produits toxiques organiques.

Received September 13, 1988
Accepted February 23, 1989
(J9883)

Reçu le 13 septembre 1988
Accepté le 23 février 1989

Nutrition is an important factor modulating the toxicity of organic xenobiotics in mammals and fishes (Campbell and Hayes 1974; Mehrle et al. 1977; Reddy et al. 1980; Bailey et al. 1982). Both direct and indirect evidence suggest that nutritional status often alters xenobiotic toxicity by influencing the activities of hepatic xenobiotic-metabolizing enzymes such as cytochrome P-450-dependent monooxygenases (MOs) and glutathione *S*-transferases (GSTs) (Campbell and Hayes 1976; Stott and Sinnhuber 1978; Sparnins et al. 1982; Ankley and Blazer 1988). Dietary factors known to affect these enzymes in mammals include deficiencies and/or qualitative differences in a variety of micro- and macronutrients (Sachan 1975; Sikic et al. 1977; Siddick et al. 1980; Wade et al. 1985).

One dietary component that has received considerable attention in mammalian toxicological studies is lipid. Dietary lipids are important, not only from an energetic standpoint, but also in terms of membrane structure, a significant factor influencing the activities of membrane-bound enzymes such as cytochromes P-450 (Bosterling and Stier 1983). Rats fed diets deficient in lipid exhibited marked decreases in hepatic MO activity (Marshall and McLean 1971; Norred and Wade 1972; Wade et al. 1985). The degree of unsaturation of dietary lipid also affects xenobiotic metabolism. Rats fed diets containing highly saturated fatty acids had lower hepatic MO and GST activities than those fed rations high in unsaturated fatty acids (Marshall and McLean 1971; Norred and Marzuki 1984; Wade et al. 1985).

Placement of the terminal double bond in dietary unsaturated fatty acids also influences the activities of xenobiotic-metabolizing enzymes. When rats were fed lipids rich in *n*-3 series fatty acids (e.g. menhaden oil), hepatic MO and GST activities were elevated compared with levels of activity found in animals fed lipids high in *n*-6 series fatty acids (e.g. corn oil) (Century 1973; Wills 1980; Dharwadkar et al. 1986). The relative importance of degree of unsaturation versus the content of specific fatty acid families (i.e. *n*-3, *n*-6, *n*-9 series fatty acids) in determining the activities of xenobiotic-metabolizing enzymes is difficult to evaluate. It is clear, however, that both factors can influence xenobiotic metabolism in mammals.

There is little information concerning possible effects of dietary lipid on xenobiotic-metabolizing enzymes in fishes. Studies with rainbow trout (*Oncorhynchus mykiss*) have shown that the addition of cyclopropenoid fatty acids in the diet of the fish can significantly affect hepatic MO, GST, and epoxide hydrolase activities (Eisele et al. 1978, 1983). Ankley and Blazer (1988) found that diets with different lipid profiles influenced both MO and GST activities in channel catfish; however, other factors (e.g. ascorbic acid content) also varied among the diets used in that study. Definitive information concerning the effects of dietary lipid on xenobiotic metabolism in fishes is essential because fish foods routinely used in toxicological and drug metabolism research can vary greatly in lipid composition, especially if obtained from commercial sources.

In the present study, channel catfish (*Ictalurus punctatus*) were fed diets containing three different types of lipid: menhaden oil (MHO), soybean oil (SBO), or beef tallow (BFT). MHO and SBO both are high in polyunsaturated fatty acids; however, MHO has a high percentage of *n*-3 series fatty acids, whereas SBO is rich in *n*-6 series fatty acids. BFT contains a higher percentage of saturated fat and *n*-9 series fatty acids than either MHO or SBO. Previous work with channel catfish has shown that all three types of dietary lipid result in similar growth rates and are adequate for the maintenance of healthy fish (Stickney and Andrews 1971; Gatlin and Stickney 1982). After feeding these diets for about 4 mo, hepatic MO and GST activities were determined, and fatty acid profiles in the liver were characterized.

Materials and Methods

Materials

Food grade SBO and BFT were obtained from Arrowhead Mills, Hereford, TX and ICN Biomedicals, Costa Mesa, CA, respectively. MHO was obtained from Zapata Haynie Corporation, Reedville, VA.[1] Concentrations of chlorinated organic contaminants in the MHO, as reported by the Zapata Haynie Corporation, were uniformly very low, including lindane, heptachlor, heptachlor epoxide, aldrin, and endrin (all not detectable), toxaphene (<0.3 mg/kg), polychlorinated biphenyls (<0.2 mg/kg), DDD (<0.2 mg/kg), DDT (0.1 mg/kg), dieldrin (0.05 mg/kg), methoxyclor (0.01 mg/kg) and chlordane (0.20 mg/kg). To minimize possible peroxidation, all lipids were stored refrigerated for less than 3 mo before use. In addition, the MHO was stored under nitrogen.

[1]Mention of trade names or commercial products does not constitute endorsement or recommendation by the U.S. Environmental Protection Agency, Food and Drug Administration or Fish and Wildlife Service.

TABLE 1. Composition of experimental diets.

Ingredient	Percentage
Vitamin-free casein	32.60
Gelatin	9.40
Dextrin	33.05
Cellulose	10.00
Lipid[a]	10.00
Mineral mix[b]	4.00
Vitamin mix[c]	0.95

[a]Menhaden oil, soybean oil, or beef tallow.

[b]Mineral mix of Williams and Briggs (1963) supplemented with cobalt chloride (1 mg/kg diet), aluminum potassium sulfate (0.7 mg/kg diet), and sodium selenite (0.08 mg/kg diet).

[c]Following given in milligrams per kilogram diet: ascorbic acid, 120; thiamin, 10; riboflavin, 20; pyridoxine, 10; folic acid, 5; Ca pantothenate, 40; choline chloride, 3000; niacin, 150; vitamin B_{12}, 0.06; retinyl acetate (500 000 IU/g), 12; alpha tocopherol, 100; cholecalciferol (1 000 000 ICU/g), 6; menadione-Na-bisulfite, 80; inositol, 400; biotin, 2.

All other diet ingredients were obtained from United States Biochemical Corporation, Cleveland, OH. Chromatography supplies, including the fatty acid methyl ester standards, GLC-10 and PUFA No. 1, were obtained from Supelco Inc., Bellefonte, PA. Sources of other reagents are described by Ankley et al. (1986).

Treatment of Fish

Fingerling channel catfish were obtained from the Cohutta Fish Hatchery and maintained in a constant flow system at the University of Georgia Whitehall Laboratory for about 4 mo. During this time fish received Purina Catfish Chow. At the beginning of the experiment, fish (mean weight 171 g, $n = 60$) were randomly allocated into six tanks (75 fish per tank) in duplicate 90% recirculating systems. Each recirculating system contained three of the tanks, to which the three experimental diets were randomly assigned. Flow rate in both systems was 11–12 L/min, and temperature was maintained at 20 ± 2°C throughout the experiment. Experimental diets were similar to the diet described by Durve and Lovell (1982), and were prepared according to standard experimental guidelines (NRC 1983). Diet compositions are given in Table 1. Diets were stored at −20°C until 1 d before use, when they were thawed at 4°C. Fish were fed 1.5% of their body weight every other day, and total amounts fed were adjusted every 2–3 wk. Fish were maintained on this regime for 116 d before sampling.

Enzyme Analyses

Eight fish were sampled from each of the tanks resulting in a total sample size of 16 fish per diet. The two recirculating systems were sampled separately on consecutive days. Fish were killed by decapitation and their livers immediately removed and minced in cold 0.15 M KCl solution. Livers were homogenized in four volumes of a cytochrome P-450-stabilizing buffer (0.1 M KH_2PO_4 at pH 7.25, 1 mM EDTA, 1 mM dithiothreitol, 20% glycerol), and microsomes and cytosol were prepared by differential centrifugation as described by Ankley et al. (1986). Tissue fractions were stored at −80°C until analysis. MO activities were assayed within 3 d of preparation, and GST activity was measured within 5 d.

The 0-dealkylation of 7-ethoxyresorufin (EROD) and 7-ethoxycoumarin (ECOD) by microsomal MOs was measured

TABLE 2. Effects of dietary lipid on percent liver weight to body weight ratio, hepatic microsomal and cytosolic protein concentrations, and total liver lipid in channel catfish. Data are given as $\bar{X} \pm$ SD and sample size was 16 for all values, except liver lipid, where the sample size was four. Values followed by different letter superscripts varied significantly ($P<0.05$) from other values in that row.

	Beef tallow	Soybean oil	Menhaden oil
Liver wt/body wt (×100)	1.40 ± 0.29^a	1.45 ± 0.32^a	1.31 ± 0.22^a
Microsomal protein (mg/g liver)	7.8 ± 0.9^a	9.1 ± 1.9^b	9.1 ± 1.6^b
Cytosolic protein (mg/g liver)	44.0 ± 6.4^a	43.7 ± 6.8^a	42.3 ± 5.4^a
Total liver lipid (%)	4.4 ± 1.0^a	3.4 ± 0.6^a	5.1 ± 1.8^a

TABLE 3. Effects of dietary lipid on hepatic microsomal EROD and ECOD activities and hepatic cytosolic GST activity. EROD activity is given as pmol resorufin formed·min^{-1}·mg microsomal protein^{-1} or as pmol resorufin formed·min^{-1}·g liver^{-1}. ECOD activity is given as pmol hydroxycoumarin formed·min^{-1}·mg microsomal protein^{-1} or as pmol hydroxycoumarin formed·min^{-1}·g liver^{-1}. GST activity is expressed as nmol 1-chloro-2, 4-dinitro benzene conjugated ·min^{-1}·mg cytosolic protein^{-1}. Data are given as $\bar{X} \pm$ SD and sample size was 16 in all cases. Values followed by different letter superscripts varied significantly from other values in that row.

	Beef tallow	Soybean oil	Menhaden oil
EROD			
per milligram protein	126 ± 30^a	103 ± 15^b	132 ± 45^a
per gram liver	972 ± 215^a	932 ± 207^a	1165 ± 324^b
ECOD			
per milligram protein	47 ± 10^a	45 ± 7^a	53 ± 15^a
per gram liver	369 ± 84^a	395 ± 84^a	469 ± 124^b
GST	853 ± 164^a	955 ± 221^a	1125 ± 317^b

using fluorimetric procedures reported by Burke and Mayer (1974) and Ullrich and Weber (1972), respectively. EROD activity in the present experiment was about four-fold higher than that found in previous studies with channel catfish in this laboratory (Ankley et al. 1986; Ankley and Blazer 1988). This was probably due to the use of a newer, and quite possibly purer, resorufin standard for calibrating activity in the present study. GST activity toward 1-chloro-2,4-dinitrobenzene was determined using the spectrophotometric method of Habig et al. (1974). MO and GST activities were assayed at 30 and 20°C, respectively. Protein concentration was determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

Lipid Analyses

Livers from four fish per diet were collected and stored at −70°C for analysis of total lipid and fatty acid composition. Total lipid was extracted from liver samples using the method of Maxwell et al. (1980). Lipid samples were weighed and converted to their respective fatty acid methyl esters by the method of Morrison and Smith (1964). The methyl esters were analyzed using a Perkin-Elmer Model 3920B gas-liquid chromatograph (GLC) equipped with a 1.8-m stainless steel column packed with 10% SP-2330 on 100/120 mesh Chromosorb W AW. GLC column and inlet temperatures were 200°C and flame ionization detector temperature was 250°C. Flow rate of helium carrier gas was 30 mL/min. Fatty acids were identified by comparison with standards and by comparison with relative retention times reported for fatty acid methyl esters injected under similar conditions.

TABLE 4. Effects of dietary lipid on the fatty acid composition of livers of channel catfish. Values are given as relative percentage of total fatty acid methyl esters, and are the $\bar{X} \pm$ SD for four fish per diet.

	Diet		
Fatty acid	Beef tallow	Soybean oil	Menhaden oil
14:0	0.4±0.3	0.2±0.1	0.5±0.1
16:0	19.7±4.5	16.5±1.0	18.4±0.5
16:1*n*-7	4.9±2.6	1.1±1.2	2.4±0.5
18:0	11.6±1.1	19.0±2.0	15.4±1.6
18:1*n*-9	39.4±3.0	27.4±3.8	31.1±3.8
18:2*n*-6	1.1±0.6	5.0±2.4	0.7±0.2
18:3*n*-3	0.1±0.1	0.2±0.1	0.1±0.1
20:1*n*-9	2.9±0.5	2.3±0.7	3.0±0.2
20:2*n*-6	1.9±0.2	0.1±0.1	0.2±0.1
22:0	2.3±1.2	5.3±1.9	0.7±0.2
20:3*n*-3	7.8±2.1	3.3±1.0	0.3±0.2
20:4*n*-6	4.2±2.3	9.3±0.6	1.6±0.4
22:1*n*-9	0.2±0.1	0.1±0.1	0.1±0.2
20:5*n*-3	0.2±0.1	0.2±0.2	4.0±0.1
22:4*n*-6	0.2±0.1	0.1±0.1	0.3±0.1
24:1*n*-9	1.6±1.0	3.9±0.6	0.6±0.2
22:5*n*-3	0.2±0.2	0.8±0.3	2.3±0.1
22:6*n*-3	1.4±1.0	5.4±1.9	18.4±2.0
Σ saturated	33.9±2.5	41.0±0.9	35.1±2.1
Σ monounsaturated	48.9±4.0	34.8±5.0	37.2±4.1
Σ polyunsaturated	17.0±6.5	24.2±4.2	27.7±2.1
Σ *n*-9	44.0±1.5	33.7±3.8	34.8±3.7
Σ *n*-6	7.4±3.2	14.4±2.8	2.7±0.2
Σ *n*-3	9.7±3.3	9.8±1.4	25.0±2.0
n-3/*n*-6 ratio	1.4±0.2	0.7±0.1	9.3±0.5

Statistical Procedures

Enzyme data were analyzed using a randomized block design with diet as the main factor and sampling date/recirculating system as the blocking variable. Total lipid data were analyzed using one-way analysis of variance. When analysis of variance indicated a significant effect, multiple comparisons among the diet groups were made using Duncan's multiple range test. Results were considered significant at $p<0.05$.

Results

There were no disease outbreaks, nor was there any differential mortality among the diet groups during the study. There were no significant differences in growth among the diet groups. Final mean body weights ($\pm$ SD) for 18 fish from the BFT, SBO, and MHO groups were 300.7 ± 104.1, 318.4 ± 117.9, and 311.6 ± 109.7, respectively.

Relative liver weight, cytosolic protein concentration, and total lipid content did not vary significantly among the diet groups (Table 2). Microsomal protein concentration, however, was significantly affected by diet (Table 2). It was about 15% higher in fish fed the SBO and MHO diets than in those fed the BFT ration.

Hepatic microsomal EROD activity was significantly affected by diet, and was 20–30% higher in fish fed the BFT and MHO diets than in those receiving the SBO diet (Table 3). Hepatic microsomal ECOD activity exhibited a similar trend;

however, there were no statistical differences among the diet groups (Table 3). Because microsomal protein concentration varied significantly (Table 2), we also examined differences among the diet groups when EROD and ECOD activities were normalized to liver weight (Table 3). When normalized to liver weight, hepatic EROD and ECOD activities were significantly higher in fish fed the MHO diet than in those fed the SBO or BFT rations. This difference was primarily due to the lower levels of microsomal protein in the BFT group relative to the MHO and SBO groups.

Hepatic cytosolic GST activity also varied significantly among the diet groups (Table 3). Levels of GST activity in fish fed the MHO ration were approximately 20 and 30% higher than in animals receiving the SBO and BFT diets, respectively.

In general, liver fatty acid profiles reflected the content of the dietary lipid sources (Table 4). The livers of fish fed the BFT, SBO, and MHO diets were rich in *n*-9, *n*-6 and *n*-3 fatty acids, respectively. Total monounsaturated fatty acid content was considerably higher in the livers of fish fed the BFT diet than in those fed the SBO and MHO diets; these differences were mainly related to the content of 18:1*n*-9. Total polyunsaturated fatty acid content was similar for the SBO and MHO groups, and slightly lower in the BFT group. Major differences existed among the groups in the *n*-6 and *n*-3 fatty acid composition of the liver. The total *n*-6 fatty acid content for the SBO group was two-fold greater than that of the BFT group and six-fold greater than that of the MHO group. The total *n*-3 fatty acid content was similar for the BFT and SBO groups, but was nearly three-fold higher in fish fed the MHO diet. While the *n*-3/*n*-6 fatty acid ratios for the BFT and SBO groups were near equality, this ratio exceeded nine for the MHO group.

Discussion

Numerous studies have shown that dietary lipid markedly affects xenobiotic metabolism in mammals; however, little is known concerning the effects of lipid on xenobiotic metabolism in fishes. To our knowledge, this is the first report of the effects of common dietary lipids on xenobiotic metabolism in fishes. Catfish fed MHO had significantly higher MO activity than fish fed SBO when results were expressed on a microsomal protein basis, and when MO activity was normalized to liver weight, MHO-fed animals had higher levels of MO activity than fish receiving either BFT or SBO. Cytosolic GST activity also was significantly higher in fish fed MHO than in those fed SBO and BFT. Certain types of contaminants can induce MO and GST activities in mammals and fishes; however, given the small concentrations of contaminants present in the MHO, this is an unlikely explanation for our observations.

The higher MO and GST activities observed in fish fed MHO, compared with those fed SBO, were both qualitatively and quantitatively similar to observations made in mammalian studies comparing diets rich in *n*-3 versus *n*-6 series fatty acids. Century (1973) reported that hepatic MO activity toward hexobarbital and aminopyrine was 20–30% higher in rats fed rations containing MHO than in those fed corn oil. Wills (1980) found that MO-mediated metabolism of aminopyrine and benzo[*a*]pyrene was 20–30% higher in the livers of rats fed herring oil than in those fed corn oil. Dharwadkar et al. (1986) reported that hepatic microsomal cytochrome P-450 concentration and ECOD activity, and cytosolic GST activity toward 1-chloro-2,4-dinitrobenzene, were higher in rats fed MHO than in those fed corn oil.

Various hypotheses are available to explain variations in MO activities between animals fed *n*-3 and *n*-6 series fatty acids. The consensus of most researchers is that qualitative differences in fatty acid content of the microsomal membrane somehow influence the catalytic efficiency of cytochromes P-450 (Wills 1980; Dharwadkar et al. 1986; Wade et al. 1986). Although the exact mechanism by which *n*-3 and *n*-6 series fatty acids affect MO activity is uncertain, we believe that it is noteworthy that the influence of these fatty acid families on MO activity is similar in mammals and fishes.

MO activity was higher in fish fed BFT than in those fed SBO when activity was normalized to microsomal protein; however, there was no difference between the dietary groups when activity was expressed on a liver weight basis. Century (1973) found no significant difference in hepatic MO activity between groups of rats fed BFT and corn oil, whereas Wills (1980) observed that MO activity was higher in rats fed corn oil than in those fed lard. Other studies showed that MO and GST activities were higher in rats fed higher unsaturated vegetable oil (corn oil) than in animals fed diets rich in saturated vegetable oil (coconut oil) (Wills 1980; Norred and Marzuki 1984; Wade et al. 1985). Because of differences in the types of lipids used, direct comparison of the trends observed in our study with those in mammalian studies is not possible. However, it is apparent that both degree of unsaturation and position of the terminal double bond of dietary fatty acids are important factors influencing the regulation of xenobiotic-metabolizing enzymes in mammals.

Hepatic MOs, and to a lesser extent GSTs, are inducible via exposure of the organism to a variety of organic xenobiotics. Treatment of fishes with environmental contaminants such as polychlorinated biphenyls and polycyclic aromatic hydrocarbons has been shown to increase hepatic MO and GST activities (Andersson et al. 1985; Ankley et al. 1986). Several mammalian studies have indicated that the relative inducibility of hepatic MO and GST activities is strongly influenced by dietary lipid (Marshall and McLean 1971; Wade et al. 1985, 1986). Thus, we might have seen even greater differences in MO and GST activities in this study had we compared the relative inducibility of these enzyme systems in fish fed the different lipids.

Previously we reported that hepatic MO activity was higher in channel catfish fed a laboratory-prepared diet compared with those fed a commercial ration (Ankley and Blazer 1988). The laboratory diet had much higher levels of *n*-3 series fatty acids than the commercial ration; however, the latter diet also was deficient in ascorbic acid. Based on mammalian studies, we hypothesized that ascorbic acid deficiency may have been responsible for the observed difference in MO activity. The results of the present study, however, indicate that the fatty acid composition of the diets also may have played a role.

Differences in hepatic MO and GST activities among the three diet groups were accompanied by marked alterations in the fatty acid composition of the livers of the fish. The livers of fish fed BFT, SBO, and MHO were relatively abundant in *n*-9, *n*-6, and *n*-3 series fatty acids, respectively. These results are consistent with those of Stickney and Andrews (1971), who reported the fatty acid composition of the livers of channel catfish fed MHO and BFT. Comparable trends in the fatty acid composition of liver microsomal membrane phospholipids have been reported in mammalian studies using similar types of dietary lipid (Century 1973; Agradi et al. 1975; Wills 1980). Previous work with fish showed that differences in the fatty acid composition of total lipids versus microsomal membrane phos-

pholipids in the liver were relatively small (Farkas and Csengeri 1976). Therefore, differences in MO activities among the diet groups in the present study could have been related to alterations in the microsomal membrane, as suggested in mammalian studies (Wills 1980; Dharwadkar et al. 1986; Wade et al. 1986).

It is less apparent how dietary lipid may have influenced cytosolic GST activity. The functions of hepatic GSTs are numerous. In addition to the metabolism of organic xenobiotics, certain GSTs in mammals are involved in the reduction of organic hydroperoxides (Schramm et al. 1985). Polyunsaturated *n*-3 series fatty acids, such as those found in MHO, are particularly prone to oxidation both in the diet and in cellular membranes. Thus, it may be that the elevated GST activity observed in catfish fed MHO reflects a compensatory/adaptive response to higher levels of potentially harmful organic hydroperoxides.

In summary, our data indicate that hepatic MOs and GSTs in fishes, as in mammals, are affected by dietary lipids. This suggests that the type of lipid fishes receive in the diet could influence their sensitivity to organic xenobiotics metabolized by MOs and GSTs. Moreover, dietary lipid also could affect the metabolism and subsequent disposition of drugs commonly used in fish culture. Thus, our observations are of practical significance to researchers working with fishes in the areas of aquatic toxicology and drug metabolism and disposition.

Acknowledgments

This work was partially supported by the Cooperative States Research Service USDA agreement No. 87-CRSR-2-3040 and by Federal Hatch Forestry Research grant No. 25-26-GC295-001. Zapata Haynie Corporation generously supplied the MHO. Thanks are extended to the Department of Fisheries and Wildlife at Michigan State University for support of G.T.A. during portions of this work. We also thank Dr. M. Agosin for the use of laboratory facilities for the enzyme assays, and Drs. A. E. Wade, J. McKim, and J. Nichols for their critical appraisal of this manuscript.

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Mechanism and Occurrence of Focal Scale Resorption among Chum Salmon (*Oncorhynchus keta*) of the North Pacific Ocean

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Bigler, B. 1989. Mechanism and occurrence of focal scale resorption among chum salmon (*Oncorhynchus keta*) of the North Pacific Ocean. Can. J. Fish. Aquat. Sci. 46: 1147–1153.

A recently documented scale characteristic of chum salmon (*Oncorhynchus keta*) originating from Hokkaido, Japan, has been found distributed almost exclusively among stocks of Asian origin. Scales from 10 544 chum salmon collected from 25 near-shore locations throughout the North Pacific Ocean were examined. Resorption of the scale focus, including instances where an easily visible hole had been formed, was found among 10.8% of Japanese chum salmon and 15.9% of chum salmon originating from the Soviet Union. In North America, this trait was infrequent (<0.5%) among populations north of the Aleutian Islands, British Columbia, and Puget Sound, and was not found in Cook Inlet, Prince William Sound, and Southeast Alaska. Focal scale resorption arises from osteoclastic cellular activity, most likely following the first winter of life, and remains as a permanent mark thereafter. This unique, uniformly occurring, easily identifiable scale characteristic will provide a valuable tool for stock identification. Evidence of focal scale resorption is also reported in sockeye (*O. nerka*), coho (*O. kisutch*), and king salmon (*O. tshawytscha*), but was not found in pink salmon (*O. gorbuscha*), sampled from locations in Alaska.

Une caractéristique des écailles du saumon kéta (*Onchorhynchus keta*) qui se retrouve presque exclusivement parmi les stocks asiatiques, a été découverte récemment à Hokkaïdo. On a examiné les écailles de 10 544 saumons kétas provenant de 25 stations côtières de tout le Pacifique-Nord. La résorption du noyau de l'écaille, allant parfois jusqu'à l'apparition d'un trou bien net, se manifeste chez 10,8 % des kétas japonais et 15,9 % des kétas soviétiques. En Amérique du Nord, ce trait est rare (<0,5 %) parmi les saumons vivant au nord des Aléoutiennes, en Colombie-Britannique et dans le golfe de Puget, et n'a pas été découvert dans le passage de Cook, le golfe du Prince-William et le sud-est de l'Alaska. L'écaille se résorbe par suite d'ostéoclase cellulaire, vraisemblablement après le premier hiver, et cette marque demeure permanente par la suite. Ce trait caractéristique, uniforme, et facile à identifier constitue un outil précieux pour l'identification des stocks. On signale également la présence de ce trait chez les saumons sockeye (*O. nerka*), coho (*O. kisutch*) et chinook (*O. tshawytscha*), mais pas chez le saumon rose (*O. gorbuscha*), en Alaska.

Received October 4, 1988
Accepted March 9, 1989
(J9895)

Reçu le 4 octobre 1988
Accepté le 9 mars 1989

Removal of material from the focal area of scales such that a hole is formed has recently been described among chum salmon (*Oncorhynchus keta*) of Hokkaido, Japan, and the Bering Sea (Bigler 1988a, 1988b). This form of scale resorption ranges from small pits to removal of material from both the annual ring (osseous) scale layer and underlying fibrillary plate (composed of collagen fibrils) and can result in a hole easily visible to the unaided eye. No parameter has been found which differentiates chum salmon carrying this trait from those which do not. Focal scale resorption occurs independent of spawning population size, age class, sex, the growth-related parameters of fork length and circuli numbers (Bigler 1988a) and sexual maturity (Bigler 1988b). Confinement of damage to the first, and occasionally the second annual growth zone, and the presence of reparative scale material in some specimens has suggested formation early in life.

The author has been unable to locate any published reports which describe this scale trait among salmon. Ichikawa (1953) working with carp (*Cyprinus carpio*), and Yamada (1956) and Ikeda et al. (1974) working with goldfish (*Carassius auratus*) found that focal scale resorption resulted following protracted periods of starvation. No evidence has been found to support the hypothesis that focal resorption in chum salmon results from a reduced diet (Bigler 1988a).

Focal resorption occurs at a constant rate within a given spawning population, and populations in close geographic proximity show similarities in the frequency of occurrence (Bigler 1988c). It has not been possible to assign a fish to a specific spawning stock; however, the use of this characteristic in chum salmon stock identification may be valuable if the incidence of focal resorption between populations is sufficiently different.

This study describes the mechanism responsible for focal scale resorption in chum salmon and the distribution of this trait among chum salmon throughout the North Pacific Ocean. The distribution of focal resorption among the scales of an individual fish, the occurrence in other species of salmon, and the use of this trait as a population identification character will also be addressed.

Materials and Methods

Age is recorded in European notation where two numerals which precede and follow a decimal point, respectively, indicate the observed number of freshwater and ocean annuli on the scales.

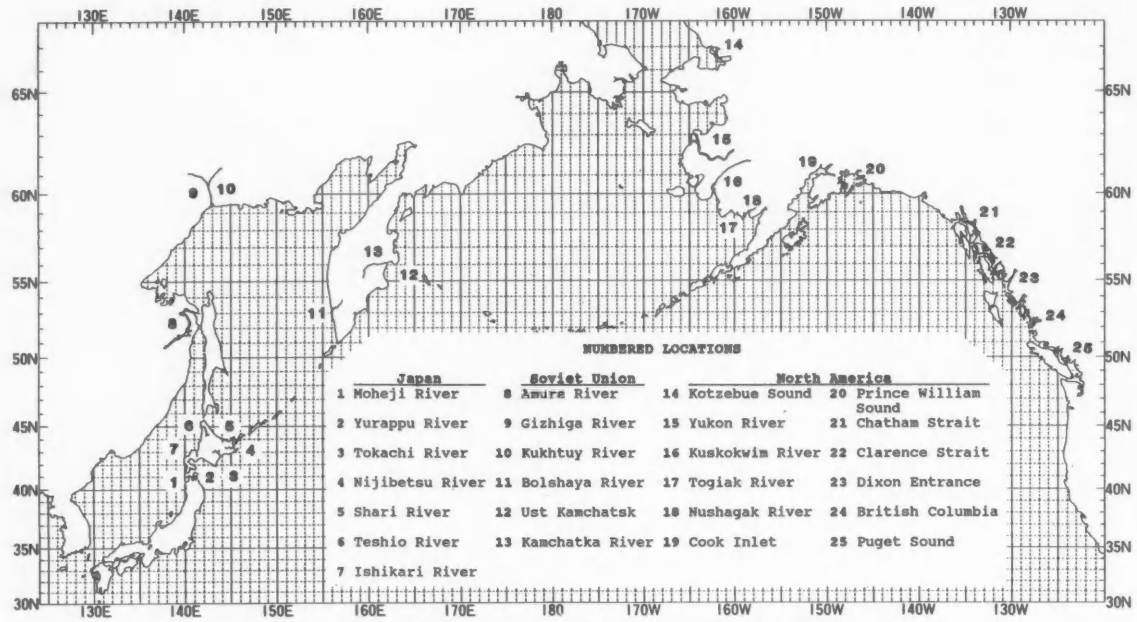

FIG. 1. Major chum salmon producing locations throughout the North Pacific Ocean sampled for focal scale resorption

Collection and Examination of Tissue Samples

Age 0.0 and 0.1 chum salmon, raised in an aquarium from a single brood, were preserved in alcohol and examined for focal scale resorption. A single individual survived through the first winter and was found to have a large number of scales which showed evidence of focal scale resorption. Skin samples were collected from this fish, embedded in paraffin using standard techniques and sectioned to 4 μm. Sections were mounted on glass slides, stained with hematoxylin and eosine, and examined under a light microscope.

Fifteen adult chum salmon were captured during the spawning migration at Moheji, Hokkaido (Fig. 1). The entire skin was removed from the left or right side (head to tail, dorsal to ventral centerline) from two fish found to have focal scale resorption. From the caudal peduncle, every fifth and sixth vertical scale rows above and below the lateral line, including the lateral line scales, were examined for scale resorption and the results mapped on graph paper. For analytical purposes, regenerated and missing scales were combined with those found without focal resorption.

Scanning Electron Microscopy

Scale impressions representing several properties of focal resorption were identified and the original scales obtained for scanning electron microscopic examination and photography. Scale readers, when examining acetate impressions, may dismiss focal resorption as tissue, debris, or regenerated scales. Photos were used to compare features associated with focal scale resorption in acetate impressions with that of the original scale, and to illustrate how this trait would appear to scale readers.

Distribution among North Pacific Chum Salmon

Not all scales collected from fish having focal resorption bear this trait (Bigler 1988a) and the degree that an individual fish must be sampled to detect this trait to acceptable statistical limits has not been tested. Accordingly, the collection of one scale per fish will most likely under estimate the occurrence of focal resorption in a population.

Scale samples were obtained from several major chum salmon rivers of the Soviet Union, Japan, Alaska, British Columbia, and Washington State (Fig. 1; Table 1). All scales were assumed to be collected from below the posterior insertion of the dorsal fin and two or three rows above the lateral line. Samples representing 1986 chum salmon returns to Japan were collected from seven hatchery locations and several scales from each fish were mounted between glass slides. Acetate impressions of one scale per fish, collected in 1986, were available from North America, with the exception of British Columbia and Puget Sound which included two scales per fish. Scale samples from locations in the Soviet Union were collected during the 1980, 1983, and 1984 seasons with all locations being sampled in 1983. Besides acetate impressions, paper packets with several scales from each fish were obtained from the Soviet Union. Two scales were mounted and examined where available from these packets.

Basic statistical procedure dictates that the percentage of fish among a population which have focal scale resorption can be deduced from a random sample. In order to discuss the distribution of this trait among populations, a measure of population size is necessary. No single measure of population size was found applicable to the Soviet Union and Japan such that these data could be combined to represent chum salmon stocks of Asian origin. Japanese data are weighted according to the number of fry produced by each hatchery facility 4 yr prior to collection, the season which accounts for the greatest number of returns in the year of collection (Hokkaido Regional Salmon Hatcheries 1983). Weighting the Japanese stocks by near-shore commercial catches is not as representative because scales were collected at hatchery sites and not from the mixed-stock com-

TABLE 1. Sample size, occurrence, and weighted percentage of focal scale resorption detected in 25 chum salmon populations sampled throughout the North Pacific Ocean. Samples were collected from mixed stock fisheries where possible.

Sample location	Sample size	Focal scale resorption		Weighted[a] %
		No.	%	
North America				
Puget Sound (1986)	964	6	0.6	0.06
British Columbia (1986)	523	5	1.0	0.15
Dixon Entrance (1986)	496	0		
Clarence Strait (1986)	494	0		
Chatham Strait (1986)	491	0		
Prince William Sound (1986)	444	0		
Cook Inlet (1986)	495	0		
Nushagak River (1986)	473	3	0.6	0.04
Togiak River (1986)	512	4	0.8	0.04
Kuskokwim River (1986)	493	8	1.6	0.05
Yukon River (1986)	1033	1	<0.1	<0.01
Kotzebue Sound (1986)	516	2	0.4	<0.01
	6934	29	0.4	0.35
Soviet Union				
Kamchatka River (1983)	181	20	11.1	
(1984)	184	26	14.1[b]	
Ust Kamchatsk (1983)	148	11	7.4	
	513	57	11.1	2.2
Bolshaya River (1980)	222	115	51.8[b]	
(1983)	154	36	23.4	
(1984)	129	49	38.0	
	505	200	39.6	10.4
Kukhtuy River (1983)	299	8	2.7	
Gizhiga River (1983)	259	26	10.0	
	558	34	6.1[c]	1.4
Amure River (1983)	299	18	6.0[b]	1.9
	1875	309	16.5	15.9
Japan				
Moheji River (1986)	223	32	14.4	0.9
Yurappu River (1986)	264	7	2.7	0.1
Tokachi River (1986)	263	23	8.7	2.9
Nijibetsu River (1986)	245	16	6.5	2.5
Shari River (1986)	212	25	11.8	2.1
Teshio River (1986)	280	18	6.4	0.4
Ishikari River (1986)	248	38	15.3	1.9
	1735	159	9.2	10.8
Total	10 544	494	4.7	—

[a]Japanese samples are weighted by the number of releases from each hatchery source 4 yr before the year of sampling. All other samples are weighted according to commercial harvest.

[b]Samples from the Soviet Union which are represented by more than one scale per fish and considered most representative of the sampled population.

[c]Data collected from these two rivers is combined for weighting purposes.

mercial catch. Though dated, commercial catch statistics cited by Shepard et al. (1968) represent the most reliable information of relative population size for the Soviet Union (Table 1). North American data are weighted by commercial catches of the fisheries where samples were collected (Alaska Department of Fish and Game, 333 Raspberry Road, Anchorage, AK 99517, USA, unpubl. data; C. Gosselin, 555 West Hastings St., Vancouver, B.C., V6V 5G3, Canada, pers. comm.; J. Sneva, 115 General Admin. Bldg. Olympia, WA 98504, USA, pers. comm.).

FIG. 2. Light micrograph of skin tissue including a portion of the upper scale which is being resorbed. Close examination of this area indicates that cells responsible are of the type which normally build scales at the margin. Areas of focal resorption (A) were found in close proximity to the margin of other scales (B), or scale pocket. Separation of tissues is an artifact of sectioning. Magnification 200×.

Occurrence among Other Salmon Species

Acetate impressions of sockeye (*O. nerka*), pink (*O. gorbuscha*), coho (*O. kisutch*), and king salmon (*O. tshawytscha*), scales were obtained from Prince William Sound, Cook Inlet (pink and coho) and the Yukon River, Alaska, respectively, and examined for focal scale resorption. An analysis among physical and population parameters will be reported elsewhere.

Results

Mechanism of Focal Scale Resorption

Focal scale resorption occurs as a result of osteoclastic cells which arise from tissues surrounding the affected scale and which resorb the fibrous and calcified scale layers (Fig. 2). Sectioned skin tissues reveal that these cells are either recruited from the scale pocket wall or from the margin of scales in close proximity to erode the osseous or fibrous scale layers at the focus.

Distribution among Scales of an Individual Fish

Scales with focal scale resorption occur in discontinuous patches over the entire body, and occur more frequently among

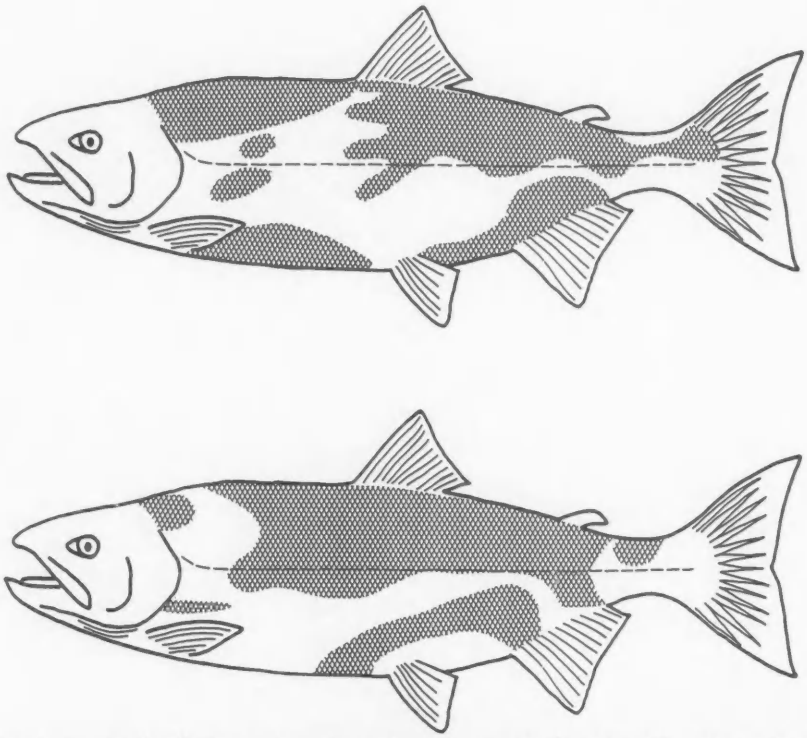

FIG. 3. Areas on the skin of two chum salmon where adjacent scales (cross-hatched areas) were found to have focal scale resorption. Patterns are concluded from examination of every fifth and sixth scale row along the lateral line from the caudal peduncle to the operculum and mapping the results on graph paper. Resorbed scales were also found isolated in areas not indicated.

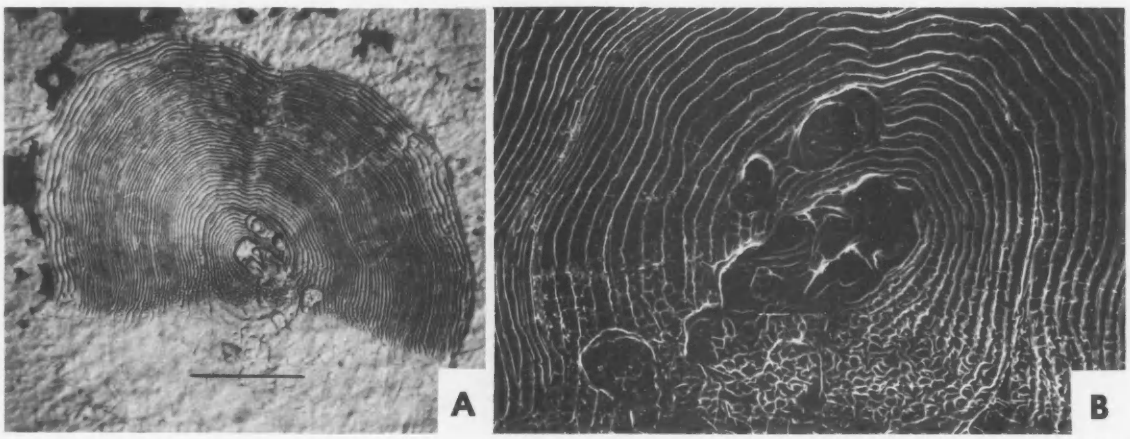

FIG. 4. (A) light micrograph of an acetate plastic impression (bar scale indicates 1 mm), and (B) scanning electron micrograph (magnification 62×), of the same chum salmon scale eroded by focal scale resorption. Ridges within the resorbed areas indicate reparation to the ossified scale layer.

scales above the lateral line (Fig. 3). By sampling every fifth and sixth vertical scale rows, approximately one-third of all scales were examined. Mapping the areas where adjacent scales were found to have focal resorption indicates that scales above the lateral line include 55.5 and 66.6% resorbed scales, versus 35.9 and 48.3%, respectively, among scales below the lateral line of the fish sampled. Few lateral line scales were found with focal resorption although neighboring scales showed such evidence.

Distribution among Chum Salmon of the North Pacific Ocean

Focal scale resorption occurs almost exclusively among chum

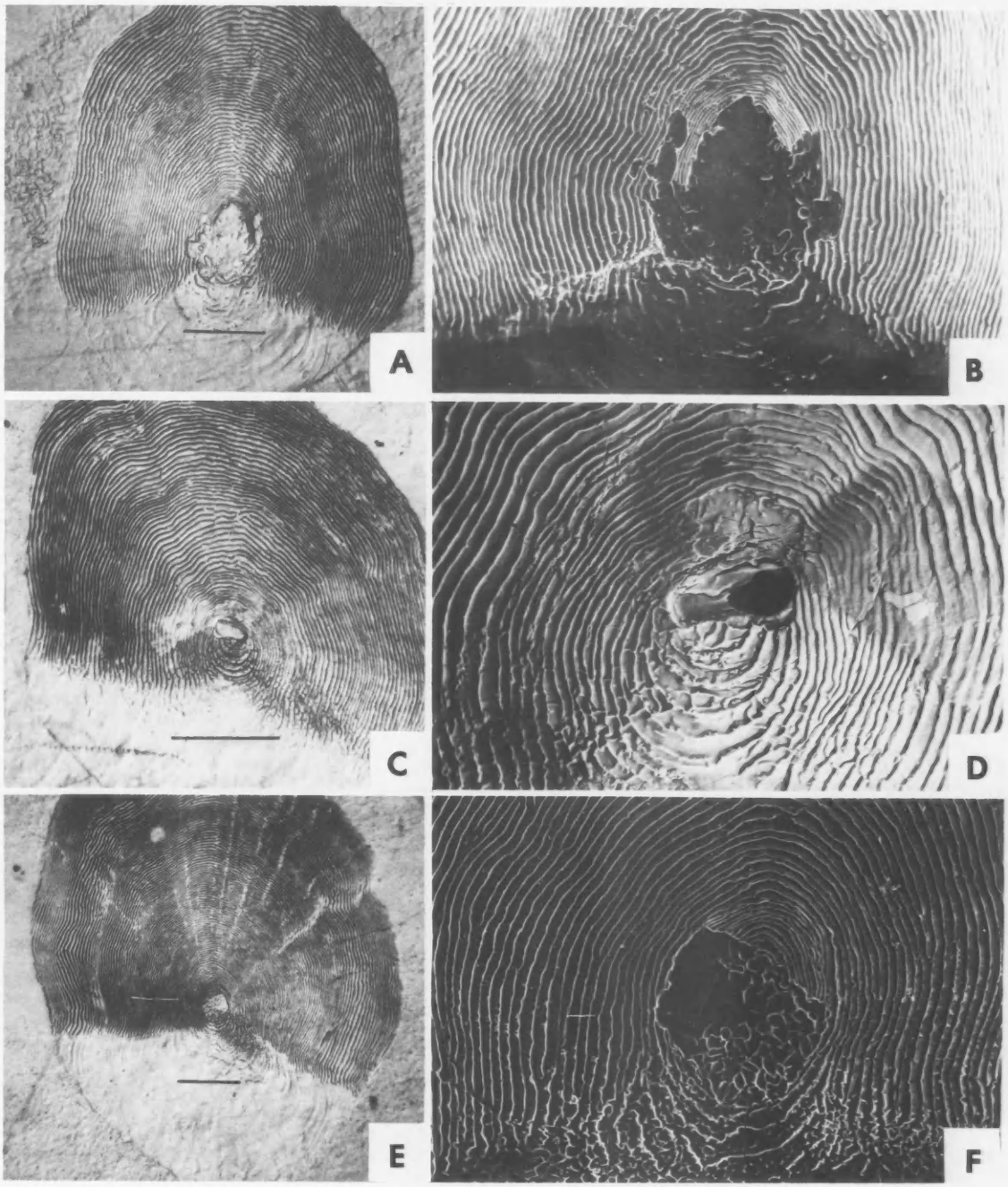

FIG. 5. Light and scanning electron micrographs of scales representative of focal scale resorption found in coho (A,B; magnification 35×), sockeye (C,D; magnification 75×), and king salmon (E,F; magnification 66×) collected from Cook Inlet, Prince William Sound, and the Yukon River, respectively. Light micrographs are taken of acetate impressions to illustrate how focal scale resorption would appear to scale readers. Bar scales indicate 0.5 mm.

salmon originating from Japan and the Soviet Union (Fig. 4; Table 1). Scales collected from 10 544 chum salmon from 25 locations throughout the North Pacific Ocean were examined. Samples collected at individual locations in Japan were found to include from 2.7 (Yurappu River) to 15.3% (Ishikari River) focal scale resorption. Soviet chum salmon were found with

2.7 (Kukhtuy River) to 51.8% (Bolshaya River) focal resorption. The distribution of focal scale resorption among North American samples ranged from none (all south central and Southeast Alaska locations) to 1.6% (Kuskokwim River). Weighted by the appropriate factor, focal resorption was found among 10.8% of chum salmon originating from Japan and 15.9% of chum salmon originating from the Soviet Union. Focal resorption was detected in 0.35% of North America chum salmon, a frequency difference of two orders of magnitude when compared with the occurrence among Asian stocks, and was not found among populations from Cook Inlet, Prince William Sound, or Southeast Alaska.

Occurrence among Other Salmon Species

Focal resorption was also found in sockeye, coho, and king salmon sampled from locations within Alaska (Fig. 5). Among coho salmon sampled from a mixed-stock drift gill net fishery in Cook Inlet, 24.3% ($n = 379$) were found to have this trait. Three percent ($n = 723$) of mixed stock sockeye salmon originating from Prince William Sound, and 40.2% ($n = 323$) of king salmon sampled from the Yukon River. Focal scale resorption was not found among pink salmon ($n = 467$) sampled from Cook Inlet, Alaska. The physical appearance of focal resorption in these species (Fig. 5) is somewhat different than that found in chum salmon (Fig. 4). Resorption resulting in a hole through both scale layers was rarely found among North American salmon species and resorbed areas were generally small.

Discussion

Developing within connective tissues of the skin, chum salmon scales increase in size as a result of cellular activity at the margin. Scale growth is related to feeding, and resorption of scales normally commences following the cessation of feeding associated with the spawning migration (Grey and Setna 1931; Major and Craddock 1962; Bilton and Robins 1971a, 1971b, 1971c; Suzuki and Wada 1985). Focal scale resorption in chum salmon initially develops after the first winter of life and remains as a permanent mark thereafter. Previous investigations (Bigler 1988a, 1988b) have found that the frequency of focal scale resorption is the same among age 0.2 through 0.5 fish, between males and females, and between sexually mature and immature fish captured at sea. The age 0.1 fish found to have focal scale resorption in this study had been kept removed from most environmental factors in filtered seawater under controlled conditions and feeding regime. Development of focal scale resorption under these controlled conditions suggests a predisposed tendency to develop this trait. None of the 0.0 age chum salmon examined ($n = 88$) showed evidence of focal scale resorption.

Cells which are responsible for resorption originate from tissues surrounding the scales. There are two hypotheses to explain the exact tissues from which these cells are recruited. First, the observation that the focus of each scale lies in close proximity to the actively growing margins of two adjacent scales supports the hypothesis that cells which are building the margin of one scale migrate through the surrounding connective tissues to resorb adjacent scales (Fig. 2). Second, cells responsible for resorption may be recruited from the "scale pocket wall" of underlying or overlying scales as these are areas which are active in the production of scale-building cells (J. Yamada, Faculty of Fisheries, Hokkaido University, 3-1-1 Minato-cho, Hakodate, 041, Japan, pers. comm.). Whichever is the case, cells responsible for resorption at the scale focus are presumed the same as those which function to enlarge the scale.

Selection for scales from below the posterior insertion of the dorsal fin and two or three rows above the lateral line increases the probability of detecting focal resorption. The mapping of areas where adjacent scales possess this trait indicated that larger numbers of scales above the lateral line were affected. Admittedly, this investigation involved a sample size of only two fish which had been selected in a biased manner (since a hand lens was used slightly resorbed scales would not have been detectable), and the mapping of contiguously resorbed scales ignores those found isolated. Various fish may show differing degrees of effects such that detection may be more a function of the number rather than the location of scales collected.

Sample sizes used in the detection of this trait among North American populations were high and it is not likely that focal resorption would have gone undetected. Samples which consisted of more than one scale (Kamchatka and Bolshaya Rivers, 1984 and 1980, respectively; Puget Sound and British Columbia) showed higher percentages of focal scale resorption than other samples represented by a single scale (Table 1). Furthermore, it is reasonable to assume that the intention of collecting samples represented by a single scale was for age or more detailed analysis. Accordingly, these samples must have been subjected to a certain scrutiny prior to pressing. These observations indicate that the number of fish found to have focal resorption is a conservative estimate where samples are limited to one scale.

This study lays the foundation for use of focal scale resorption as a tool for stock identification. Using this characteristic alone, chum salmon from the Pacific Ocean which possess this characteristic are very likely to have originated from the Asian continent. Fournier et al. (1984) and Wood and Oliver (1987) are among several authors who have recently discussed the use of discrete or continuous variables in a maximum likelihood procedure for stock identification. Fournier et al. (1984) used morphological measurements and electrophoretic data in examining several chum salmon populations of British Columbia. Sexual dimorphic differences found in that study required that male and female data were analyzed separately. Wood and Oliver (1987) found it necessary to confine the maximum likelihood process to one age class when using variables related to body size. The inclusion of focal resorption in a predictive model would not require consideration of sexual or age/growth related differences within any given spawning population. By using the presence or absence of focal scale resorption in conjunction with other variables which may or may not be influenced by sexual or age differences the probability of successfully identifying Asian chum salmon may be enhanced.

Acknowledgments

The author performed portions of this study under a scholarship granted by the Japanese Ministry of Education (Monbusho) and through cooperation with the Alaska Department of Fish and Game. I sincerely thank Dr. Juro Yamada, Head of the Laboratory of Physiology and Ecology, Hokkaido University, and Mr. Chuck Meacham of the Alaska Department of Fish and Game for their assistance and encouragement. A debt of gratitude is also extended to Mr. Benjamin Van Allen, Alaska Department of Fish and Game, Mr. Camille Gosselin, Department of Fisheries and Oceans, Canada, Ms. Kate Myers, Fisheries Research Institute, University of Washington, and Mr. John Sneva, Washington Department of Fisheries, for providing scale sam-

ples. My thanks to Mr. James Wolf, Alaska Department of Public Safety Crime Laboratory, Anchorage, for his invaluable help with Scanning Electron Microscope photography.

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Growth, Mortality, and Secondary Production in Natural Populations of the Blue Mussel, *Mytilus edulis*

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Mallet, A. L., and C. E. A. Carver. 1989. Growth, mortality, and secondary production in natural populations of the blue mussel, *Mytilus edulis*. Can. J. Fish. Aquat. Sci. 46: 1154–1159.

Juveniles (17–26 mm) from 11 natural populations of the blue mussel *Mytilus edulis* were each transplanted to nine sites along the coast of Nova Scotia, Canada. Production was determined from the growth and mortality profiles of each stock over the period from November 1985 to December 1986. Stock and stock by season explained 26.6% of the variance in production whereas site and site by season accounted for 24.1%. Stock and its interactions explained most of the variance in mortality, while site and its interactions were more important in explaining the variance in growth. Growth, rather than mortality, was the primary determinant of production.

De jeunes moules bleues (*Mytilus edulis*) de 17 à 26 mm de long, issues de 11 populations naturelles, ont été transplantées dans neuf sites sur la côte néo-écossaise. La production a été mesurée à partir des profils de croissance et de mortalité de chaque stock entre novembre 1985 et décembre 1986. Le stock, et le stock en fonction de la saison, expliquent 26,6 % de la variance de la production, tandis que le site, et le site en fonction de la saison, en expliquent 24,1 %. Le stock et les interactions où il joue expliquent la majeure partie de la variance de la mortalité, tandis que le site et ses interactions intervenaient plus dans la variance de la croissance. La croissance est le premier déterminant de la production, avant la mortalité.

Received May 24, 1988
Accepted March 9, 1989
(J9744)

Reçu le 24 mai 1988
Accepté le 9 mars 1989

The heterogeneity in secondary production values observed among populations of a species has commonly been explained in terms of variable ecological and environmental interactions. Specifically, in situ measurements of production are strongly influenced by factors such as competition, predation, or temperature (Dare 1976; Downing 1984). In contrast, production estimates based on physiological calculations reflect the maximum production potential of the species (Bayne and Worrall 1980) but exclude the effects of ecological interactions and natural mortality. The present study compares the production of mussel populations (*Mytilus edulis*) grown in suspension culture where predation levels are negligible, competition for food is reduced and separate cohorts are easily distinguishable. Estimates obtained under these relatively "ideal" conditions represent the realized production potential of the species, i.e. increase in weight minus losses due to natural mortality over time.

Genetic differences in metabolic efficiencies and tolerance ranges may explain part of the variability in secondary production values. In order to separate this genetic component, the growth and mortality profiles of different genetic groups must be obtained under similar environmental conditions (e.g. Ayles and Baker 1983; Dickie et al. 1984; Mallet et al. 1986). Previous studies on the blue mussel (*Mytilus edulis*) have shown that: (1) geographically isolated populations are genetically variable (Levinton and Suchanek 1978; Mallet et al. 1987a,b), (2) the magnitude of the genetic effect varies depending on the stage of the life cycle (Diehl and Koehn 1985; Mallet et al. 1986), and (3) interactions between the genotype and the environment may be significant (Mallet et al. 1987a,b). Although these studies emphasize the genetic variation among stocks, they do not assess the implications of this variation for production.

This study compares the growth and mortality profiles of eleven stocks each grown at nine sites in Nova Scotia, Canada. From these data we determine: (1) the relative importance of genetic versus environmental factors in determining growth, mortality, and production and (2) the relative effects of growth and mortality on production.

Materials and Methods

Experimental Design

Juvenile mussels (less than 5 mo old) from 11 natural populations were transplanted to nine locations along the coast of Nova Scotia, Canada (Fig. 1). Details of the juvenile collection and transplantation are presented in Mallet et al. (1987a,b). Note that because of the scarcity of juveniles, stock K was transplanted to only four of the nine sites (2,3,7, and 9) and stock F to all sites except 8. Loss of the mortality cages at site 7 meant that data were available from only nine of the original 10 sites (see Mallet et al. 1987a).

To determine growth rates, 425 mussels were transferred into 75-cm long tubular nylon sleeves with a 40-mm mesh size. One hundred and ten of these experimental units, 10 from each of the 11 stocks, were deployed at each of the nine sites, or 990 units over all sites. At each sampling event, October 23, 1985 (initial), April 15, 1986 (winter), July 4, 1986 (spring), September 4, 1986 (summer), and December 1, 1986 (fall), we removed two of the 10 sleeves from each stock at each site.

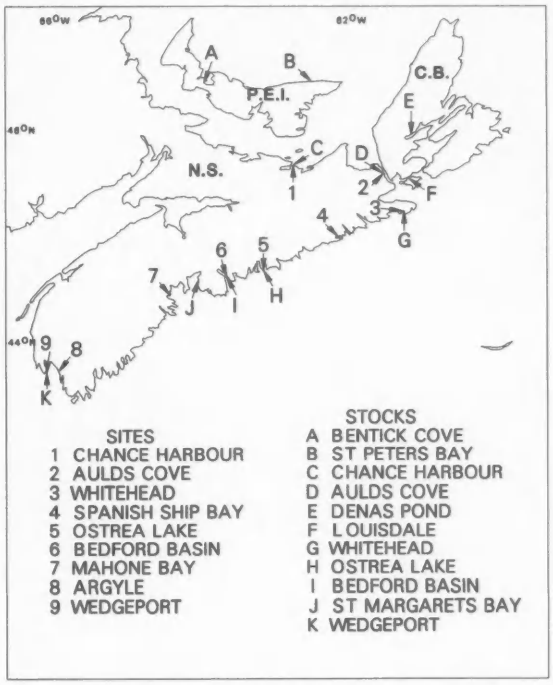

FIG. 1. Locations of the 11 stocks (A–K) and nine sites (1–9) used in this study. P.E.I. is Prince Edward Island; N.S. is Nova Scotia; and C.B. is Cape Breton. The Northumberland Strait is located between P.E.I. and N.S.

TABLE 1. Initial least squares mean (u) and standard error (SE) for shell length and whole weight of each stock averaged over all sites.

	Shell length		Whole weight	
Stock	u (mm)	SE	u (g)	SE
A	24.5	0.47	0.45	0.01
B	26.4	0.47	0.30	0.02
C	21.7	0.47	0.40	0.01
D	23.0	0.47	0.30	0.01
E	19.1	0.47	0.30	0.00
F	22.2	0.50	0.46	0.02
G	17.3	0.47	0.36	0.02
H	25.7	0.47	0.31	0.01
I	18.2	0.47	0.38	0.01
J	20.6	0.47	0.42	0.01
K	19.0	0.72	0.42	0.02

Shell length and whole dry weight (shell plus tissue) were measured on a random sample of 40 mussels, i.e. 20 from each of two sleeves at each sampling event. Seasonal growth rates were calculated from the change in the average weight or shell length of each stock at each site over time between each sampling event. Final mean shell length and whole weight were adjusted for initial differences with an analysis of covariance.

To estimate mortality, two replicates of one hundred and fifty juveniles from each stock at each site were initially placed in separate 2-mm Vexar cages of approximately 700 ml volume. Twenty-two cages (11 stocks by two cages per stock) were deployed at each site, or 198 cages over all sites. At each sampling event, the mussels in each cage were counted and the number standardized to 125 (April), 100 (July), or 50 (September), respectively. The change in the number of animals between sampling events represented the seasonal mortality rate for each stock at each site (see Mallet et al. 1987b).

Production values were calculated by the method of Allen (1951). For each stock at each site we plotted the number of survivors against mean dry weight for each sampling interval. Production was calculated as the average number of animals present during an interval multiplied by the average weight increment over that interval.

Statistical Analysis

A variance component analysis (maximum likelihood procedure; Spector and Goodnight 1987) was used to estimate the contribution of the random effects (stock, site, season, and their interactions) to the variance in growth, mortality, and production. These numeric values provide indices of genotype, environment, and genotype by environment effects. The following cross-classified random model was used:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \alpha\gamma_{ik} + \beta\gamma_{jk} + \epsilon_{ijk}$$

where μ is the population mean common to all observations, α, β, and γ are the stock, site, and season main effects with their respective second degree interactions, and ϵ is the error term. All random terms are assumed to be normally distributed with a mean of 0 and a variance σ^2.

The relative effects of growth and mortality on production were assessed by means of a path analysis (Li 1975). Path coefficients are standardized partial regression coefficients and their magnitude is an index of the importance of a given relationship. These coefficients also provide a means of assessing the separate contribution of growth and mortality to the variance in production values. The regression coefficients were obtained from an analysis of covariance where the regression effects were first adjusted for the main factors (Searle 1971). The path coefficients were determined by multiplying these regression coefficients by the appropriate ratio of the standard deviations.

Results

Initial Characteristics

Initial shell length and whole weight for each stock are shown in Table 1. Part of the variability among stocks at the onset of the experiment can be explained by the asynchrony in settlement time. For example, the stocks with greater shell length, A,B,C,D, and H settled in late June as compared with late July for most of the other stocks.

Effect of Stock

Adjusted estimates of mean shell length, whole weight, mortality, and production of each stock over all sites are shown in Table 2. Growth averaged over all stocks and sites was 28 mm in shell length and 3.8 g in whole dry weight. The variation in growth among stocks was substantial, but the rankings of the stocks were similar for shell length and whole weight ($r = 0.81$, $p = 0.002$). The stocks originating from the Northumberland Strait area (A to D) had the highest whole weight, whereas the Cape Breton stocks (E and F) showed the least weight gain. Because stock K was grown at only four of the nine sites, three

TABLE 2. Least squares means of final shell length, whole dry weight (g), mortality (%), and production (g) for each stock over all sites, after adjustment for inital differences. SE is the standard error; Dev, the average performance of a given stock measured as a deviation from the mean of all stocks.

	Shell length			Whole weight			Mortality			Production		
Stock	*u* (mm)	SE	Dev	*u* (g)	SE	Dev	*u* (%)	SE	Dev	*u* (g)	SE	Dev
A	51.5	1.0	1.9	5.9	0.3	1.7	14.2	2.2	−4.9	4.9	0.5	1.5
B	50.1	1.3	0.5	4.8	0.3	0.6	13.6	3.5	−5.5	3.8	0.4	0.4
C	50.3	0.8	0.7	4.4	0.2	0.2	8.7	2.1	−10.1	3.8	0.4	0.4
D	53.1	0.9	3.5	5.3	0.2	1.1	13.2	1.5	−6.1	4.2	0.5	0.8
E	44.6	1.0	−5.0	2.7	0.3	−1.5	56.7	11.0	37.6	1.5	0.3	−1.9
F	45.4	0.9	−4.2	3.6	0.2	−0.6	39.3	6.3	19.9	2.4	0.3	−1.0
G	52.9	1.3	3.3	4.2	0.3	0.0	20.5	4.6	1.4	3.3	0.3	−0.1
H	52.0	1.2	2.4	4.3	0.3	0.1	16.4	2.9	−2.7	3.4	0.3	0.0
I	46.9	1.2	−2.7	3.1	0.3	−1.1	9.1	1.4	−10.0	2.8	0.4	−0.6
J	47.2	0.9	−2.4	3.3	0.2	−0.9	11.6	1.7	−7.5	2.8	0.3	−0.6
K	51.9	1.4	2.3	4.8	0.3	0.6	7.0	1.4	−12.1	4.0	0.1	0.6

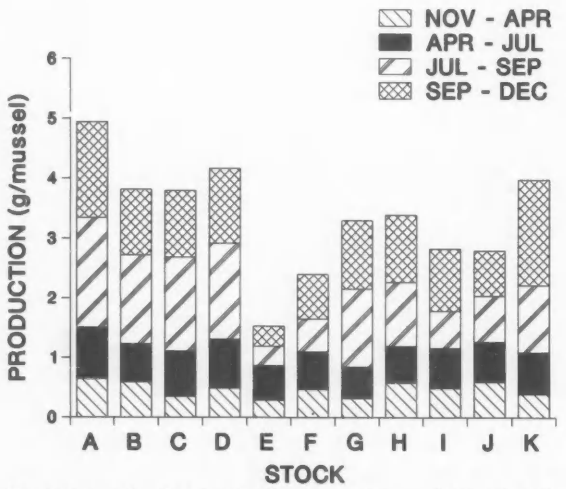

FIG. 2. Seasonal and total production (grams per mussel) for each stock averaged over all sites.

of which were above average, its performance is likely an overestimate. Stock accounted for a smaller proportion of the total variance in growth than did site and season (Table 4).

Averaged over all sites, stocks E and F exhibited the highest mortality, 57 and 39%, respectively, whereas the other nine stocks showed mortalities less than 20% (Table 2). Most of the variance (53%) was accounted for by the stock, stock by site, and stock by season effects (Table 4). The importance of the interaction terms suggests that the stocks were reacting differently to certain site specific or season specific conditions (see Mallet et al. 1987b).

Annual production values for each stock over all sites are illustrated in Fig. 2. Among stocks, annual production estimates varied from 1.5 to 4.9 g per individual (Table 2), with the most productive stocks originating from the Northumberland Strait area (except stock K). As expected, stocks with either high mortality (E and F), or poor growth (I and J) showed the lowest production levels. Stock alone accounted for 11.6% of the variance in production (Table 4).

Effect of Site

Final shell length, whole weight, mortality, and production of each site over all stocks is shown in Table 3. Sites on the northeastern coast of Nova Scotia (1 to 4) showed generally the highest growth rates. Differences in food quantity and quality among sites may explain some of the variation in growth performance. For example, the most productive site (6) had a continual supply of nutrients from organic pollution and thus relatively high phytoplankton levels (Platt 1975). On the other

TABLE 3. Least squares means of final shell length, whole weight, mortality, and production of each site over all stocks after adjustment for initial differences. SE is the standard error; Dev, the average performance of a given site measured as a deviation from the mean of all sites.

	Shell length			Whole weight			Mortality			Production		
Site	*u* (mm)	SE	Dev	*u* (g)	SE	Dev	*u* (%)	SE	Dev	*u* (g)	SE	Dev
1	50.8	0.8	1.2	5.2	0.2	1.0	28	6.3	8.4	3.8	0.4	0.5
2	54.3	0.7	4.7	4.7	0.2	0.5	18	5.4	−1.5	3.8	0.3	0.5
3	52.3	0.7	2.7	4.3	0.2	0.1	14	3.0	−5.6	3.6	0.3	0.3
4	53.8	0.8	4.2	4.5	0.2	0.3	15	3.4	−4.6	3.7	0.3	0.4
5	44.5	0.8	−5.1	2.3	0.2	−1.9	12	1.6	−7.6	1.7	0.1	−1.6
6	50.1	0.9	0.5	6.1	0.2	1.9	29	8.2	9.4	4.8	0.5	1.5
7	50.5	0.7	0.9	3.9	0.2	−0.3	20	8.2	0.4	3.0	0.3	−0.3
8	48.7	0.8	−0.9	4.1	0.2	−0.1	20	8.0	0.4	3.1	0.4	−0.2
9	41.6	0.8	−8.0	3.1	0.2	−1.1	20	5.8	0.4	2.5	0.3	−0.8

TABLE 4. Variance component analysis where DF indicates the degrees of freedom, Var is the variance component, and Per is the percent of the total variance explained by each factor.

		Shell length		Whole weight		Mortality		Production	
Source	DE	Var	Per	Var	Per	Var	Per	Var	Per
Stock	10	0.000	0.0	0.021	5.6	24.8	16.6	0.034	11.6
Site	8	0.099	0.8	0.039	10.6	0.0	0.0	0.025	10.4
Season	3	2.846	21.7	0.098	26.5	0.6	0.4	0.057	24.0
Stock × site	80	0.000	0.0	0.000	0.0	33.0	22.2	0.002	2.0
Stock × season	30	2.567	19.5	0.057	15.5	21.2	14.2	0.051	15.6
Site × season	24	4.139	31.5	0.050	13.5	6.1	4.1	0.046	13.2
Error	240	3.482		0.104		63.1		0.080	

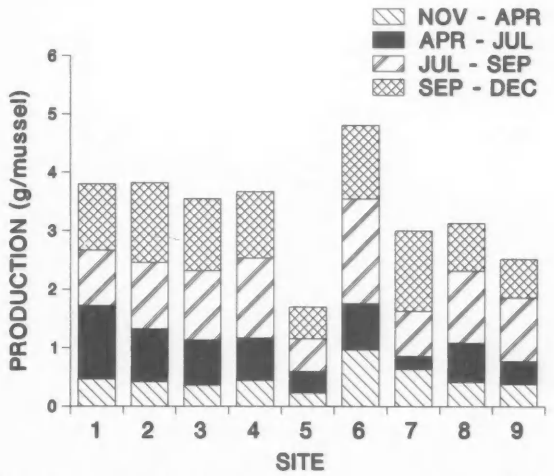

FIG. 3. Seasonal and total production (grams per mussel) for each site averaged over all stocks.

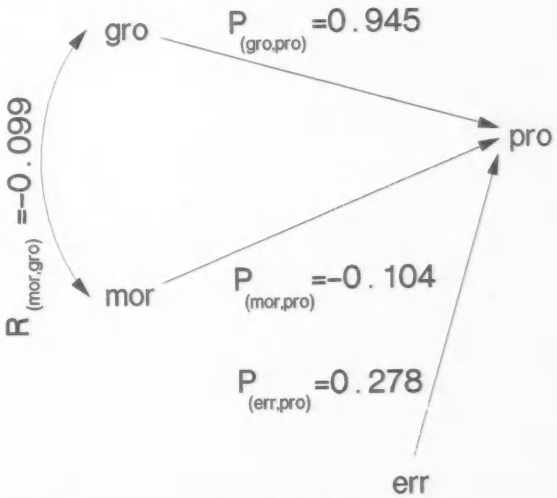

FIG. 4. Path diagram representing the relationship between production (pro); growth (gro) and mortality (mor); and the residual (err). The magnitude of the path coefficients (P) is a measure of the importance of the path. The correlation between growth and mortality ($R_{gro,mor}$) is small and negative.

hand, the least productive sites were characterized by either low water exchange, i.e. poor food supply (site 5), or high sediment loading, i.e. poor food quality (sites 8 and 9). Site alone accounted for 10.6% of the variance in growth, double that explained by stock.

Averaged over all stocks, the sites with the highest mortalities were sites 6 (29%) and 1 (28%); all the other sites had less than 20% mortality. The variation in annual mortality among sites and seasons was negligible, less than 1% (Mallet et al. 1987b). Site alone explained none of the variance in mortality.

Annual production values for each site averaged over all stocks are illustrated in Fig. 2. Among sites, production values ranged from 1.7 to 4.8 g/mussel (Table 3). Excluding site 6, the highest production levels were observed at sites along the northeastern shore of the province (sites 1 to 4). Site alone accounted for 8.5% of the variance in production values but the stock by site component was negligible (Table 4).

Effect of Season

Both growth and production exhibited a pronounced seasonal variation (Table 4). The average seasonal growth values were: 0.50 g for the winter; 0.75 g for spring; 1.23 g for the summer; and 1.24 g for fall. Whereas shell weight increased during every season, negative tissue growth rates were observed in the summer, probably due to spawning. The seasonal production values for each stock and each site are illustrated in Figs. 2 and 3, respectively. Season alone accounted for a substantial fraction of the variance in growth (32%) and production (24%) and its interaction terms were equally important.

Path Analysis

The path diagram summarizes the relationships between growth, mortality, and production (Fig. 4). The correlation between growth and mortality was significant and negative ($r = -0.099$; $p = 0.005$). The partial determination of production by growth and mortality is as follows: growth determines 88% of the variance in production, mortality determines 1%, and growth and mortality jointly determine 1.9%. The path coefficient from the residual component to production was .2784, that is, the residual accounts for only 7.7% of the variance in production.

Discussion

The variation in production among mussel populations grown under similar environmental conditions suggests that genetic

differences may be important in determining production rates. Given that genetic differences exist at the micro- and macrogeographical scales (Levinton and Suchanek 1978), genetic variation may therefore explain part of the variability in production levels observed under natural conditions. It is interesting to note that stocks from the same geographic zone showed similar production potential: stocks A to D (Northumberland Strait) have the highest production; stocks E and F (Cape Breton) the lowest; and stocks G to J (Atlantic area), an average production (stock K was omitted because of its absence at several sites). The factors responsible for this geographical pattern, for example, common founding stock or adaptation to similar environmental conditions, are obscure at this stage.

Without an environmental monitoring program, the variation in production among sites could not be correlated with specific biological or physical parameters. On a broader scale, however, the number and geographic separation of the sites provides a useful overview of the expected variability in production in Nova Scotian environments. With the exception of sites 5 and 6 which are two extreme sites, there appears to be a geographical gradient in production levels, moving from maximum production at site 1 in the Northumberland Strait to minimum production at site 9 on the southern tip of Nova Scotia. We do know that sites in the Northumberland Strait area experience higher summer temperatures than those on the Atlantic coast of Nova Scotia (sites 3 to 9). It would be interesting to investigate in depth the environmental variables that underlie this gradient.

Production levels varied considerably among seasons, being lowest in the winter (November to April). This variance in production is likely due to the timing of certain seasonal events, such as the development of the spring phytoplankton bloom, or the onset of spawning. Other environmental variables such as temperature which varies considerably among seasons may also have contributed to the strength of the seasonal effect. Although growth may vary more with season than does mortality, increased levels of mortality in the summer have been reported (Incze et al. 1980) and were observed in this data set (Mallet et al. 1987b). The importance of the site by season component indicates that sites differ in their production patterns across seasons; to control for this variation, estimates of production should always involve an annual time frame.

Variations in growth rate were explained mostly by site or season effects. This is consistent with the observation of Widdows et al. (1984) that environmental, rather than genetic, factors are primarily responsible for the physiological differences among populations. However, we did note differences in growth rate among stocks; for example, stocks A to D from the Northumberland Strait area had above average growth rates, due mostly to their heavier shell and, to a lesser extent, their above average tissue weights (Mallet et al., unpubl. data). Because stocks A to D also had lower mortality rates, their overall production values were among the highest. On the other hand, stocks E and F had below average growth rates, and these coupled with very high mortality rates accounted for their low production values. The analysis of mortality variations in these natural populations was treated in depth by Mallet et al. (1987b); it was shown that populations display both genetic variability and physiological flexibility as a means of coping with variable environments.

In suspension culture the size structure of the population changes very rapidly; animals typically attain 50–60 mm within 18 mo of settlement (Rodhouse et al. 1985). Although Dare (1976) recorded similar growth rates (length and weight) in Morecambe Bay, U.K., intertidal mussel populations generally take 4–5 yr to reach 50 mm (Bayne and Worrall 1980; Thompson 1984; Gardner and Thomas 1987) and exhibit higher losses due to mortality. Note that although the shell and tissue growth observed by Dare (1976) was comparable with our estimates, his mortality values, and thus production/biomass (P/B) values, were generally higher (1.1–3.4). This observation illustrates the great sensitivity of the P/B calculation to differences in mortality (Allen 1971). For example, our calculations of the P/B values for each stock demonstrated that those stocks with the highest mortality (E and F) had the highest P/B values; in other words P/B was negatively correlated with production performance. We conclude that while P/B may be a convenient method of comparing the turnover time of different populations or species, it is not an appropriate index of performance. Stocks E and F had very high mortality rates, presumably owing to their inability to cope with new environments, and these coupled with below average growth rates accounted for their overall low production values.

The path diagram suggests that production was more closely correlated with growth than with mortality. The relative importance of growth is perhaps not surprising considering the generally low mortality rates of mussels between 2 and 20 mo old (Freeman and Dickie 1979) and the high growth rates in suspension culture. Factors such as the stage of the life cycle and environmental conditions may influence the strength and direction of the associations among the variables affecting production. The quantification of these relationships provides useful insights about the dynamics of these variables. It also provides a statistical basis for further experiments; for example, knowing that somatic growth is the major determinant of production would translate into investigations to test the factors that stimulate or decrease somatic growth. Estimating these associations however necessitate appropriate initial replication. Several stocks and sites are recommended in order to avoid the possibility of choosing below average stocks that would distort this average relationship.

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Comparaison des relations trophiques de *Ruditapes philippinarum* en milieux estuarien et océanique à partir d'un modèle de croissance

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Bacher, C. et P. Goulletquer. 1989. Comparaison des relations trophiques de *Ruditapes philippinarum* en milieux estuarien et océanique à partir d'un modèle de croissance. Can. J. Fish. Aquat. Sci. 46: 1160–1170.

Cette étude porte sur l'établissement des relations trophiques de *Ruditapes philippinarum* en zone estuarienne et océanique du bassin de Marennes-Oléron au cours de l'année 1985–1986. L'utilisation de l'analyse en composantes principales permet de décrire, à partir des axes principaux, les fluctuations des paramètres environnementaux. Les variations de poids sec sont expliquées à partir d'un modèle de croissance allométrique utilisant les axes principaux de l'analyse en composantes principales. Les croissances observées et simulées sont supérieures en site océanique. Une déplétion de nourriture apparaît en période de floraison phytoplanctonique automnale entre les deux sites. Les remises en suspension du sédiment, provoquant de fortes turbidités, limiteraient la croissance en zone estuarienne. Les problèmes de la stratégie d'échantillonnage et de l'échelle temporelle des relations trophiques sont posés à partir des résultats du modèle.

A comparison of trophic relationships for *Ruditapes philippinarum* in estuarine and oceanic areas: the use of a growth model. Trophic relationships in *Ruditapes philippinarum* were studied in estuarine and oceanic areas of Marennes-Oleron during 1985 and 1986. Principal components analysis (P.C.A.) was used to reveal the fluctuations of the descriptors from the principal axes. Changes in dry weight were predicted in each area through an allometric growth model and were related to the principal axes derived from the P.C.A. The results of the model were used to compare the growth in each area and to point out the descriptors responsible for the discrepancies, such as autumnal blooms or silt resuspension. Attention was paid to the sampling strategy related to the time scale of the phenomena accounting for trophic relationships.

Reçu le 25 mai 1988
Accepté le 17 février 1989
(J9757)

Received May 25, 1988
Accepted February 17, 1989

La palourde japonaise *Ruditapes philippinarum*, introduite dans les années 1970, fait actuellement l'objet d'une aquaculture en plein essor sur le littoral français (600 t en 1987). En Charente-Maritime, l'activité s'est particulièrement développée depuis 1980, suite à la mise au point des techniques d'élevage dans les marais ostréicoles (Peyre *et al.* 1980). Toutefois, en raison d'une capacité trophique limitée des claires ostréicoles (Goulletquer *et al.* 1988), les densités d'élevage restent limitées à 500 g/m^2. Une nouvelle expansion de cet élevage nécessite de s'orienter vers les bassins conchylicoles et d'étudier les relations trophiques en fonction des sites de nature estuarienne ou océanique.

L'étude expérimentale des réponses physiologiques des suspensivores a permis de montrer que les taux de croissance sont régulés par l'interaction de facteurs environnementaux, dont la qualité et la quantité de nourriture (Bayne et Widdows 1978 ; Bayne *et al.* 1987). Toutefois, peu d'études ont abordé les relations trophiques *in situ* existant entre les paramètres du milieu et l'animal (voir synthèse Héral *et al.* 1987).

Généralement, les interactions synergiques des variables environnementales compliquent l'interprétation de l'importance de chaque facteur du milieu (Page et Hubbard 1987). Fréchette et Bourget (1985a) mettent en évidence les différences de croissance chez *Mytilus edulis* selon la position dans la colonne d'eau et le niveau intertidal dont les effets sont interprétés en termes de déplétion de la nourriture potentielle disponible. Berg et Newell (1986) associent la variabilité des croissances annuelles de *Crassostrea virginica* à des différences de quantités moyennes de nourriture disponible. Dans le but d'affiner l'étude du couplage croissance–nourriture potentielle, il semble donc intéressant de quantifier les relations trophiques en tenant compte de la variabilité spatio-temporelle du milieu.

Récemment, l'utilisation de méthodes statistiques de type régression multiple a permis d'établir des relations trophiques à partir de descripteurs du milieu, en les classifiant par ordre d'importance (Héral *et al.* 1984 ; Page et Hubbard 1987 ; Parache et Massé 1987). L'hypothèse de la linéarité des relations était retenue dans le cadre de ces études.

La présente étude a pour but d'étendre ces relations au cas de non-linéarité par une modélisation des variations du poids sec de l'animal, à partir des observations hydrologiques et sédimentaires du milieu durant deux années. Les propriétés du modèle permettent de classifier les variables du milieu en fonction de leur effet sur la croissance de *Ruditapes philippinarum* en zone estuarienne et océanique. Une approche prédictive permet également de quantifier l'effet du temps d'immersion sur les performances de croissance.

Lieu d'expérimentation et méthodes

L'étude des relations trophiques de *Ruditapes philippinarum* a été effectuée sur deux sites du bassin de Marennes-Oléron

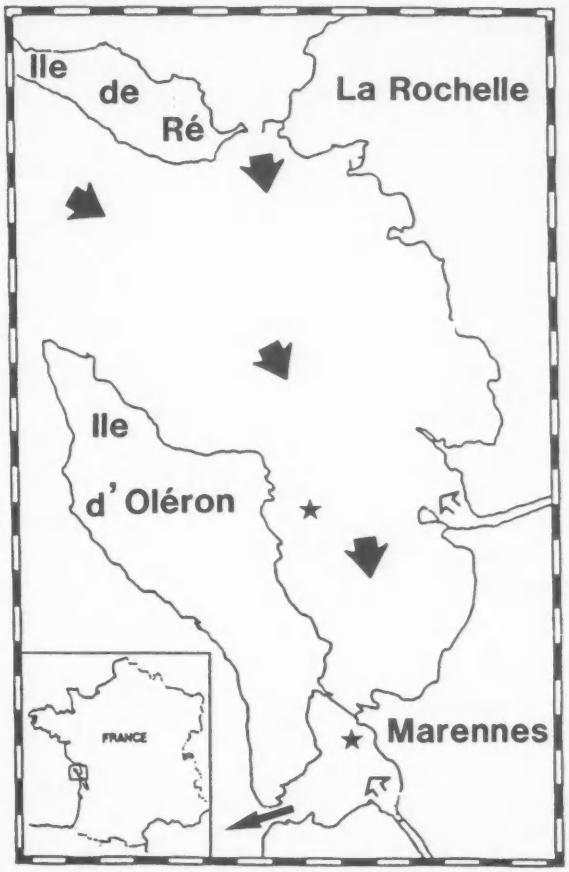

FIG. 1. Localisation des sites expérimentaux (★) et circulation globale des masses d'eaux océaniques (◆) et fluviales (◇) dans le bassin de Marennes-Oléron.

(fig. 1) : sous influence océanique, dans la partie nord du bassin, le site de Lilleau a pour substrat d'élevage un sédiment de type sableux (3 % pélites) et un temps d'immersion journalier moyen de 21 h. Le second site, au substrat de type vaseux (90 % pélites) et à temps d'immersion de 18 h est localisé près de l'Ile de Nole, dans la partie médiane du bassin ostréicole en zone estuarienne. Les vitesses de courant sont par ailleurs du même ordre de grandeur sur les 2 sites (30 à 40 $cm \cdot s^{-1}$). La circulation générale des eaux d'origine océanique et estuarienne (fig. 1) dans le bassin suit une résultante nord–sud, avec un temps de résidence de l'ordre de 5 à 9 d en fonction des oscillations tidales. Afin d'étudier les relations trophiques de l'espèce, les suivis portent sur les fluctuations de la croissance de l'animal, des paramètres hydrologiques et de l'interface eau–sédiment. Les paramètres organiques sédimentaires peuvent en effet représenter une nourriture potentielle pour les palourdes selon Bodoy et Plante-Cuny (1984) et Plante-Cuny et Bodoy (1987).

a) Stratégie d'échantillonnage

Pour mettre en relation la production du mollusque et les variables hydrologiques et sédimentaires du milieu, il est nécessaire d'obtenir la meilleure représentation possible du milieu. Si, pour l'animal, des mesures mensuelles ou bimensuelles des productions tissulaires permettent d'intégrer les fluctuations du milieu, le problème reste de définir un intervalle de temps et une stratégie d'échantillonnage appropriée au couplage benthos–pelagos (Héral *et al.* 1987).

La variabilité observée sur les paramètres hydrologiques en zone estuarienne est de quatre ordres : la stratification entre la surface et le fond de la colonne d'eau ; le cycle journalier dû à la marée ; le cycle mensuel de mortes eaux et de vives eaux ; la tendance saisonnière.

La stratégie adoptée vise à estimer les moyennes mensuelles non biaisées des variables considérées : l'échantillonnage hydrologique s'effectue en surface et au fond de la colonne d'eau, à proximité des zones d'élevage avec un intervalle de temps de 1 h 30 min pendant un demi-cycle de marée, en période de vives eaux et de mortes eaux. Une précision de l'ordre de 5 % de la moyenne journalière est ainsi obtenue avec cet intervalle de temps (Héral *et al.* 1989).

Les paramètres sédimentaires sont estimés à partir de prélèvements effectués sur chaque site d'élevage en période de vives eaux et de mortes eaux. Trois prélèvements sont réalisés sur la surface considérée afin de tenir compte de la variabilité spatiale.

b) Paramètres du milieu

En hydrologie, neuf paramètres sont retenus afin de caractériser la masse d'eau : température (Temp), saturation en oxygène (HO_2P), protéines (HPRO), lipides (HLIP) et carbohydrates particulaires (HCAR), chlorophylles *a* (HCHL) et phéopigments (HPHE), seston minéral (HPIM) et organique (HPOM). Les prélèvements et les analyses sont effectués selon les protocoles décrits par Héral *et al.* (1984).

L'étude du sédiment est effectuée par raclage du premier centimètre. Six paramètres sont retenus pour caractériser la nourriture potentielle : quantité de matière organique (SPOM), chlorophylle *a* (SCHL), phéopigments (SPHE), protéines (SPRO), lipids (SLIP) et carbohydrates particulaires (SCAR). Les protocoles sont décrits en détail par Héral *et al.* (1984).

c) Population d'élevage

Les animaux de taille comparable ($\overline{L}$ = 27,5 mm, $\overline{Pds}$ = 4,73 g) sont semés sur les deux sites en avril 1985 à une densité de 250 ind./m^2 sur une surface de 1 000 m^2. Le suivi de croissance des palourdes, réalisé jusqu'en juillet 1986, est estimé par des échantillons bimensuels ou mensuels de 20 individus prélevés au hasard dont le poids sec de chair est obtenu par lyophilisation pendant 24 h.

L'émission des gamètes, n'étant pas explicable dans le cadre de l'étude des relations trophiques, nous amène à considérer deux périodes de croissance : la classe 1 d'avril à octobre 1985 et la classe 2 de novembre 1985 à juillet 1986.

Traitement statistique

Analyse factorielle

Les interactions existant entre les variables hydro-sédimentaires descriptives du milieu ne permettent pas de préciser l'influence de chaque variable sur la croissance (tableau 1). L'explication de celle-ci nécessite par conséquent la recherche de variables explicatives synthétiques et indépendantes. L'utilisation de l'analyse factorielle permet de normaliser et d'identifier des groupes de variables corrélées

TABLEAU 1. Matrice de corrélation entre les 15 variables. Hydrologie : HPRO = protéines, HLIP = lipides, HCAR = carbohydrates, HPIM = seston minéral, HPOM = seston organique, HCHL = chlorophylle *a*, HPHE = phéopigments, TEMP = température, HO_2P = pourcentage de saturation en oxygène. Sédiment: SPOM = matière organique, SCHL = chlorophylle *a*, SPHE = phéopigments, SPRO = protéines, SLIP = lipides, SCAR = carbohydrates.

	Hydrologie									Sédiment					
	H Pro	H Lip	H Car	H Pim	H Pom	H Chl	H Phe	Temp	HO_2P	S Pom	S Chl	S Phe	S Pro	S Lip	S Carb
H Pro	1,000														
H Lip	0,211	1,000													
H Car	0,852	0,110	1,000												
H Pim	0,804	0,031	0,915	1,000											
H Pom	0,654	0,038	0,824	0,890	1,000										
H Chl	0,009	0,061	−0,026	−0,279	−0,256	1,000									
H Phe	0,536	0,132	0,674	0,514	0,517	0,345	1,000								
Temp	−0,482	−0,071	−0,548	−0,627	−0,445	0,448	−0,025	1,000							
HO_2P	−0,326	−0,140	−0,366	−0,472	−0,490	0,288	−0,098	0,533	1,000						
S Pom	0,098	0,018	0,036	−0,020	−0,104	0,021	0,073	−0,025	0,034	1,000					
S Chl	0,092	0,276	−0,121	−0,195	−0,269	0,322	0,118	0,216	0,017	0,680	1,000				
S Phe	0,097	0,040	0,009	−0,018	−0,076	0,175	0,138	0,027	−0,133	0,829	0,772	1,000			
S Pro	0,043	0,029	−0,005	−0,046	−0,114	0,015	0,016	0,025	0,030	0,969	0,709	0,815	1,000		
S Lip	0,106	0,047	0,012	−0,035	−0,123	0,035	0,029	0,020	0,064	0,980	0,720	0,815	0,980	1,000	
S Car	0,099	0,182	−0,016	−0,052	−0,166	0,045	0,051	−0,036	0,067	0,905	0,710	0,714	0,883	0,918	1,000

entre elles, indépendants les uns des autres. Ainsi, à partir de 15 séries de 41 observations sur l'ensemble des deux sites, sept axes indépendants sont retenus en fonction de leur part respective de variance totale expliquée. Une nouvelle matrice est générée par les coordonnées des observations projetées sur les axes, résumant 95 % de l'information de départ. Le taux de croissance (G) n'est pas pris en compte dans les calculs de l'analyse en composantes principales mais projeté en variable illustrative (Conan et Comeau 1986). Ceci permet de préciser les périodes et sites de forte croissance.

Modèle de croissance

Les fluctuations de l'environnement sont intégrées par l'animal au niveau du poids sec et du taux de croissance ; ainsi un modèle allométrique est utilisé, où l'exposant et le taux sont optimisés.

À la suite de Sebens (1982), les deux paramètres G (taux de croissance) et b (coefficient d'allométrie) du modèle

$$(1) \quad \frac{dW}{dt} = G \cdot W^b$$

sont considérés respectivement comme fonction du milieu et de l'espèce. En intégrant à milieu constant (G = cst, $b \neq 1$) et après correction de la fraction journalière d'immersion (Δt), on obtient :

$$(2) \quad W(t) = (W(t-1)^c + G(t) \cdot \Delta t \cdot c)^{1/c}$$

avec $t = 1$ à N (N = nombre d'observations) et $c = 1 - b$

Nous prenons comme hypothèse que la période de croissance est représentée par le temps de filtration égal à la durée d'immersion.

Le taux de croissance G est expliqué par les axes principaux X_i issus de l'analyse factorielle. La relation est écrite :

$$(3) \quad G(t) = \sum_i a_i X_i(t) + a_o$$

où a_i représente la sensibilité de chaque coefficient.

TABLEAU 2. Pourcentage de variance expliquée par les sept axes principaux de l'Analyse en composantes principales.

Axes	% Variance	Val. propres
1	35,007	5,25
2	30,134	4,52
3	11,752	1,76
4	7,384	1,11
5	4,933	0,74
6	3,445	0,52
7	2,197	0,33

Le modèle est une extension de la régression orthogonalisée (Tomassone *et al.* 1983) à un cas de non-linéarité. Les paramètres sont estimés par optimisation à l'aide de l'algorithme du Simplex (Nelder et Mead 1965 ; Schnute 1982), basée sur la minimisation du critère qui représente l'erreur résiduelle (ER)

$$(4) \quad \text{ER} = \sum_t (W_{obs}(t) - W_{cal}(t))^2$$

La validité du modèle s'effectue par comparaison du critère à la somme des carrés totale (ST) en tenant compte du nombre de degrés de liberté de chaque terme.

$$(5) \quad ST = \sum_t (W_{obs}(t) - \overline{W})^2, \text{ où } \overline{W} \text{ est la moyenne des } W_{obs}(t)$$

$$F = \frac{(ST - \text{ER})/p}{\text{ER}/(N-p)} \text{ suit approximativement}$$

une loi de Fisher F (p, $N-p$) où p est le nombre de paramètres estimés et N le nombre d'observations.

Les axes principaux X_i étant indépendants, le calcul de leur contribution c_i permet de les trier selon leur ordre d'importance dans l'explication du taux de croissance G ($c_i = a_i^2 \lambda_i$, λ_i étant la nième valeur propre).

De nouvelles optimisations sont réalisées après le retrait successif des axes les moins importants. Deux modèles successifs sont comparés à l'aide du rapport :

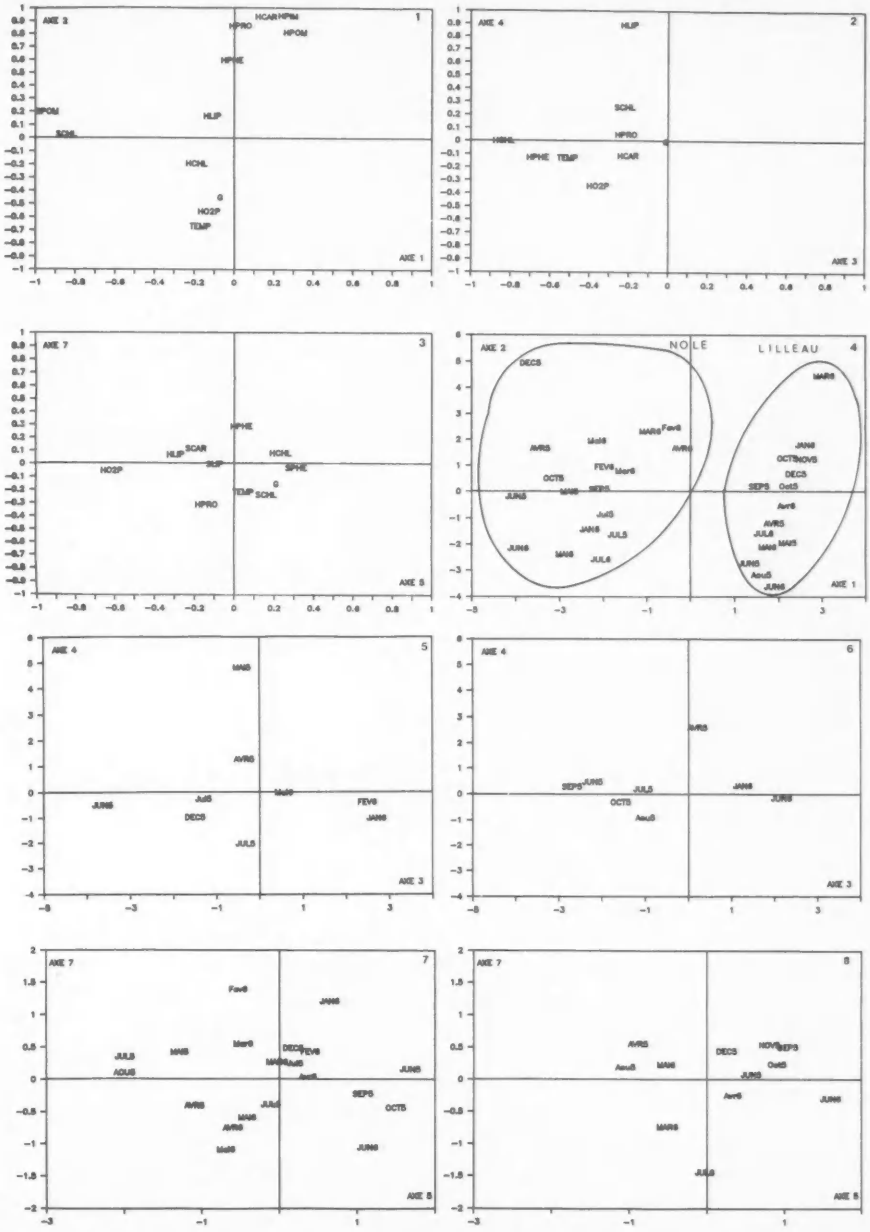

FIG. 2. Projection des descripteurs et observations sur les axes 1, 2, 3, 5 et 7 de l'Analyse en composantes principales (seuls les points bien représentés sont projetés). (1) à (3) : Corrélations entre les descripteurs et les axes. *G* : Taux de croissance en variable illustrative projetée. Hydrologie : HPRO = protéines, HLIP = lipides, HCAR = carbohydrates, HPIM = seston minéral, HPOM = seston organique, HCHL = chlorophylle *a*, HPHE = phéopigments, TEMP = température, HO_2P = pourcentage de saturation en oxygène. Sédiment : SPOM = matière organique, SCHL = chlorophylle *a*, SPHE = phéopigments, SPRO = protéines, SLIP = lipides, SCAR = carbohydrates. (4) à (8) : Projections des observations. Pour les axes 3, 4, 5 et 7, chaque site est représenté séparément. Première classe sur le site de Nole : AVR5 = avril, MAI5 = mai, JUN5 = juin, Jul5 = début Juillet, JUL5 = fin juillet, AOU5 = août, SEP5 = septembre, OCT5 = octobre 1985. Deuxième classe sur le site de Nole : DEC5 = décembre 1985, JAN6 = janvier, Fev6 = début février, FEV6 = fin février, Mar6 = début mars, MAR6 = fin mars, Avr6 = début avril, AVR6 = fin avril, Mai6 = début mai, MAI6 = fin mai, JUN6 = juin, JUL6 = juillet 1986. Première classe sur le site de Lilleau : AVR5 = avril, MAI5 = mai, JUN5 = juin, JUL5 = juillet, Aou5 = début août, AOU5 = fin août, SEP5 = septembre, Oct5 = début octobre, OCT5 = fin octobre 1985. Deuxième classe sur le site de Lilleau : NOV5 = novembre, DEC5 = décembre 1985, JAN6 = janvier, Fev6 = début février, FEV6 = fin février, Mar6 = début mars, MAR6 = fin mars, Avr6 = début avril, AVR6 = fin avril, MAI6 = mai, JUN6 = juin, JUL6 = juillet 1986.

TABLEAU 3. Corrélations entre les variables et les axes principaux de l'Analyse en composantes principales, (*G* : taux de croissance projeté en variable illustrative).

Axes		1	2	3	4	5	6	7
Variable supplémentaire	*G*	−0,083	−0,510	−0,012	−0,007	0,210	−0,032	−0,157
Variables hydrologiques	H Pro	0,030	0,862	−0,214	0,048	−0,140	0,219	0,312
	H Lip	−0,109	0,170	−0,196	0,891	−0,295	−0,140	0,068
	H Car	0,160	0,932	−0,204	−0,112	−0,100	0,071	0,029
	H Pim	0,229	0,938	0,044	−0,129	−0,062	−0,029	−0,083
	H Pom	0,306	0,847	−0,008	−0,130	0,038	−0,302	−0,054
	H Chl	−0,188	−0,190	−0,834	0,001	0,234	0,333	0,079
	H Phe	−0,010	0,596	−0,662	−0,126	0,037	−0,194	0,285
	Temp.	−0,169	−0,651	−0,511	−0,129	0,046	−0,437	−0,218
	HO_2P	−0,126	−0,556	−0,356	−0,343	−0,619	0,069	−0,056
Variables sédimentaires	S Pom	−0,942	0,199	0,134	−0,143	−0,066	−0,017	0,087
	S Chl	−0,844	0,028	−0,220	0,257	0,161	0,027	−0,238
	S Phe	−0,866	0,197	−0,003	−0,020	0,313	−0,016	−0,034
	S Pro	−0,945	0,157	0,150	−0,119	−0,045	−0,071	0,030
	S Lip	−0,956	0,173	0,120	−0,112	−0,098	−0,020	−0,006
	S Car	−0,912	0,519	0,098	0,040	−0,192	0,036	0,113

$$F = \frac{(ER_2 - ER_1)/(p_1 - p_2)}{ER_1/p_1}$$

où ER_i désigne l'erreur résiduelle du modèle i à p_i paramètres. F suit une loi de Fisher F (p_1, p_2). Tant que ce rapport n'est pas significatif, le modèle est considéré comme significatif. Comme la régression progressive pas à pas, dans un cas de linéarité, cette méthode permet de réduire le nombre d'axes significatifs.

Lorsque les paramètres du modèle sont déterminés, l'influence des descripteurs du milieu sur le poids final peut être estimée à travers une analyse de sensibilité à chaque coefficient a_i. Le tirage aléatoire d'une variable T de moyenne nulle permet d'écrire :

$$a_i' = a_i (1 + T),$$

où a_i' est la valeur modifiée de a_i introduite dans le modèle.

Si la distribution de T suit une loi normale, le poids final suivra également une distribution normale dont la variabilité sera estimée à partir des résultats de 50 simulations. Le pourcentage de variation du poids final estimera la sensibilité du modèle à l'axe considéré, selon la classe et le site choisis.

Le modèle est ensuite appliqué pour comparer l'effet du site à conditions initiales semblables : poids initiaux de chaque classe ou temps d'immersion égaux. Le poids final exprime alors une croissance potentielle, fonction du temps d'immersion et du poids initial.

Résultats

Analyse factorielle

L'analyse en composantes principales, effectuée à partir du logiciel LADDAD (1985) permet de conserver 94,85 % de la variabilité sur les sept premiers axes (tableau 2) dont les trois premiers représentent à eux seuls 76,89 %.

Les projections des variables du milieu (fig. 2_{1-3}) permettent d'identifier les corrélations avec les axes : l'axe 1 est représentatif de la totalité des fluctuations des variables sédimentaires (SPOM, SCHL, SPHE, SPRO, SLIP, SCAR) alors que l'axe 2 caractérise principalement l'évolution des variables hydrologiques hormis la chlorophylle *a* et les lipides particulaires (tableau 3).

La projection du taux de croissance en tant que variable illustrative permet de montrer l'influence de la température sur les périodes de forte croissance. La projection des observations (fig. 2_{4-8}) individualise les deux sites Nole et Lilleau, essentiellement par les caractéristiques sédimentaires très différentes. Le sédiment vaseux contient en moyenne 7 % de matière organique pour 1 % dans le sédiment sableux. Aux périodes de croissance, juillet à août 1985, mai à juillet 1986, s'oppose la période hivernale représentés par des amaigrissements marqués (mars 1986) et une nourriture potentielle détritique élevée (protéines et glucides particulaires) de qualité inférieure (fort seston minéral).

La comparaison des années 1985 et 1986 montre une répétition pour le site sableux de Lilleau, avec par ailleurs, une faible dispersion des observations caractérisant une stabilité du site. Le site vaseux de Nole montre une forte variabilité sur les axes 1 et 2 (décembre 1985, janvier 1986) avec une fluctuation interannuelle marquée (juin 1985 – juin 1986).

Les axes 3–4 (tableau 2) caractérisent les flux de nourriture avec les floraisons chlorophylliennes sur l'axe 3 en juin et septembre 1985 ainsi que les faibles quantités de chlorophylle en sortie d'hiver (janvier à mars 1986) (fig. 2_2, 2_5, 2_6). Les floraisons phytoplanctoniques sont de faible amplitude pour les deux sites au printemps 1986, de l'ordre de 3 $\mu g \cdot L^{-1}$ (10 $\mu g \cdot L^{-1}$ en 1985), d'où l'absence de corrélation avec les axes 3 et 4. Le site de Lilleau a la particularité de présenter une floraison automnale marquée en septembre 1985 (7 $\mu g \cdot L^{-1}$) qui n'est pas observée dans la partie centrale du bassin.

Les lipides particulaires, constituant une nourriture potentielle énergétique, sont associés à l'axe 4 qui oppose une période déficitaire en été (Nole, août 1985) à de fortes valeurs au printemps (Lilleau, avril 1985 ; Nole, mai 1985).

La saturation en oxygène est la variable la plus corrélée à l'axe 5, sur lequel les observations de période estivale, consécutives aux floraisons phytoplanctoniques, sont présentes (fig. 2_7 et 2_8). La période automnale se situe à l'opposé sur cet axe, associé à des chutes de saturation en oxygène et à de fortes teneurs en phéopigments dans le sédiment. Ces deux variables

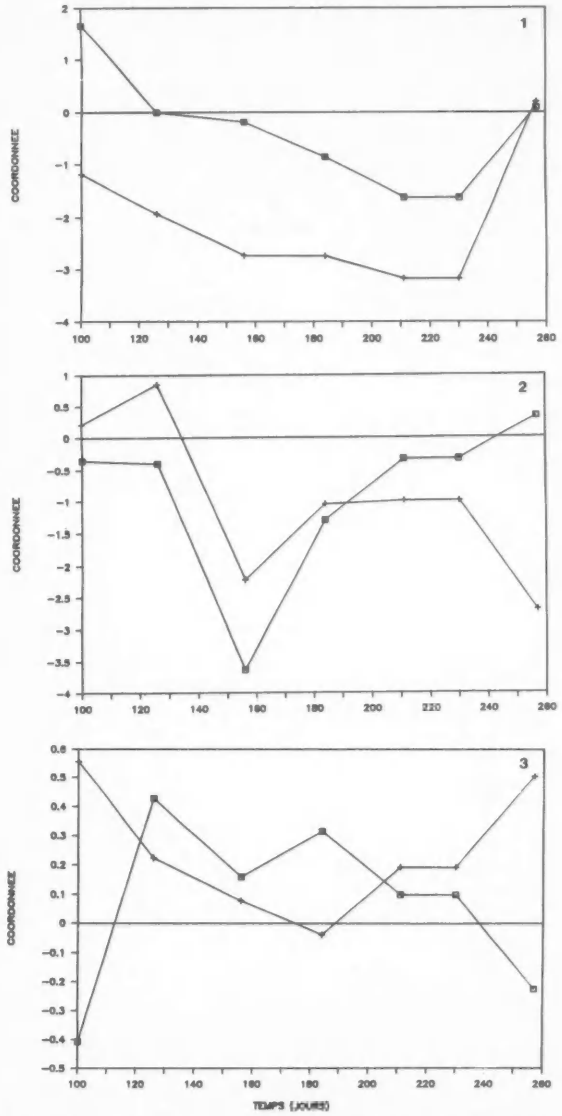

FIG. 3. Analyse en composantes principales. (1) à (3) : évolution des axes 2, 3 et 7 en fonction du temps. Les particularités des sites de Nole (□) et de Lilleau (+) pour la première période de croissance (mars–octobre) sont analysées : axe 2, différence systématique des teneurs en PIM et POM sur les deux sites ; axe 3, floraisons phytoplanctoniques comparables au printemps sur les deux sites et absente en automne sur le site de Nole (valeurs négatives des coordonnées); axe 7, opposition des teneurs en protéines faibles sur Nole et fortes sur Lilleau, aux périodes de floraisons phytoplanctoniques printanière et automnale.

indiquent une dégradation du phytobenthos dans le milieu. L'axe 5 représente par ailleurs le deuxième axe de projection du taux de croissance. L'axe 7 est représenté par les variables hydrologiques, la température, les protéines et les phéopigments, précisant des apports de nourriture ponctuels (avril, mai et juillet 1986). Les axes secondaires 5 et 7 (fig. 2_3) sont donc liés à des fluctuations saisonnières ponctuelles. Sur

TABLEAU 4. Contributions des six axes retenus, explicatifs du modèle de croissance ($a_0 = 4,968$, b = 1,735).

Axes	% Variance	a_i
2	58,30	−3,087
7	18,17	−6,383
4	11,90	2,818
5	9,28	3,0448
3	1,23	0,7178
1	1,11	−0,3961

ER = 19,85 ST = 166
n = 37 p = 8
F (8,37) = 26,46

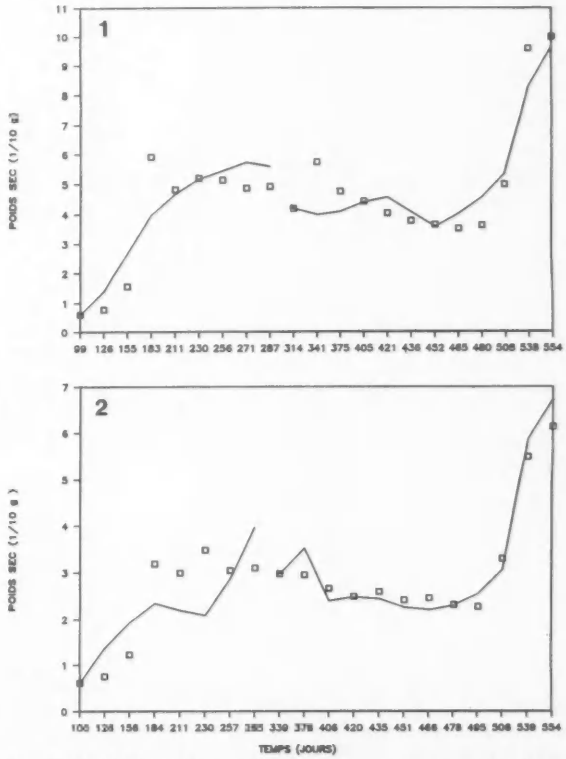

FIG. 4. Modèle de croissance fonction des axes principaux de l'ACP (ligne continue) et observations (□) pour les deux classes sur le site de Lilleau (1) et le site de Nole (2). Les axes 1, 2, 3, 4, 5 et 7 ont été sélectionnés d'après leur contribution à l'explication du taux de croissance.

l'axe 6 (tableau 3) on trouve également des apports ponctuels de nourriture (chlorophylle *a*) avec, parallèlement, une température élevée (septembre et octobre 1985). Les coordonnées des observations sur les axes 2, 3 et 7 sont projetées en fonction du temps et illustrent la différence entre sites vaseux et sableux (fig. 3).

Modèle de croissance

Les sept axes résultant de l'analyse factorielle identifient de nouveaux descripteurs synthétiques du milieu utilisés par le modèle de croissance. Un premier modèle est conçu avec les 7

TABLEAU 5. Sensibilité du poids final aux coefficients de régression des six axes retenus (valeurs absolues et pourcentage).

	1re classe				2e classe			
	Nole		Lilleau		Nole		Lilleau	
Site	Val. abs.	%	Val. abs.	%	Val. abs.	%	Val. abs.	%
Axes								
1	0,560	25,0	0,476	7,28	0,570	17,99	0,967	20,66
2	0,905	40,36	4,206	64,26	0,332	10,49	1,105	23,58
3	0,388	17,34	0,661	10,10	0,669	21,14	0,444	9,47
4	0,147	6,57	0,002	0,03	0,532	16,78	0,077	1,65
5	0,007	0,32	0,219	3,33	0,309	9,76	0,778	16,61
7	0,233	10,41	0,982	15,00	0,755	23,84	1,313	28,03

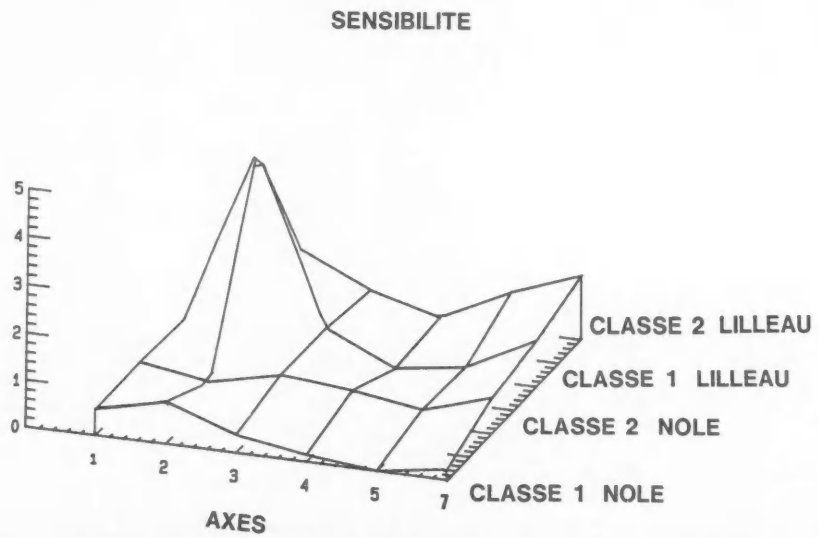

FIG. 5. Analyse de sensibilité à des fluctuations aléatoires des axes 1, 2, 3, 4, 5, et 7 retenus dans le modèle sur les sites de Nole et de Lilleau. La classe d'âge 1 est sensible à l'axe 2 (variables hydrologiques PIM, POM). La deuxième classe est sensible à l'axe 7 (protéines et phéopigments de l'eau).

axes, dont les contributions sont ensuite calculées. Les axes les moins importants en termes de contribution sont retirés un à un du modèle jusqu'à ce que la perte d'information du nouveau modèle soit significative. Les différentes simulations effectuées et la comparaison des critères obtenus (voir équation (4)) permettent de retenir finalement six axes principaux explicatifs de la croissance (tableau 4). Les contributions des axes retenus sont classées par ordre décroissant. L'axe 1, représentatif des fluctuations sédimentaires, ne peut être enlevé sans augmentation notable du critère. Les conditions hydrologiques (axe 2) constituent le principal descripteur du milieu explicatif de la croissance (58,3 %). Il est à noter que le test du bâton brisé et la comparaison des valeurs propres par rapports à 1 (Legendre et Legendre 1979) indiquent que les axes d'ordre supérieur à 4 expliquent une part négligeable de la variance totale. Cependant, l'importance prise par l'axe 7 dans le modèle (tableau 4) et son identification à des événements écologiques ponctuels montrent qu'il ne s'agit pas d'un simple bruit de l'échantillonnage.

Les optimisations et les observations sont présentées sur la figure 4. Les performances de croissance observées sont supérieures en classe 1 et 2 sur le site de Lilleau dans le nord du bassin. L'optimisation de la classe 1 du site de Nole présente des valeurs inférieures aux observations. Par ailleurs sur le site 2, deux écarts sont observés par rapport aux observations : au mois de juillet 1985, du fait d'une ponte partielle notable qui a suivi cette date, ainsi qu'au mois de décembre 1985.

L'analyse de sensibilité permet d'obtenir des informations complémentaires quant à l'importance de chaque descripteur sur la croissance des palourdes en fonction du site et des classes d'âge (tableau 5, fig. 5).

Les paramètres hydrologiques de l'axe 2 sont responsables principalement de la sensibilité de la classe 1 sur les 2 sites avec 40,36 % (Nole) et 64,24 % (Lilleau). Par contre, la sensibilité de la classe 2 est expliquée par l'axe 7 (Nole, 23,84 %; Lilleau, 28,03 %).

Par ailleurs des différences de sensibilité apparaissent selon les années et les sites et proviennent des fluctuations interannuelles et spatiales (respectivement). L'axe 1 représentant les variables sédimentaires est globalement plus important sur Nole (25 %, 18 %) que sur Lilleau (7,3 % et 21 %).

Simulations

En raison des écarts de poids initiaux importants, observés pour la 2e classe, suite à une croissance et à un effort de repro-

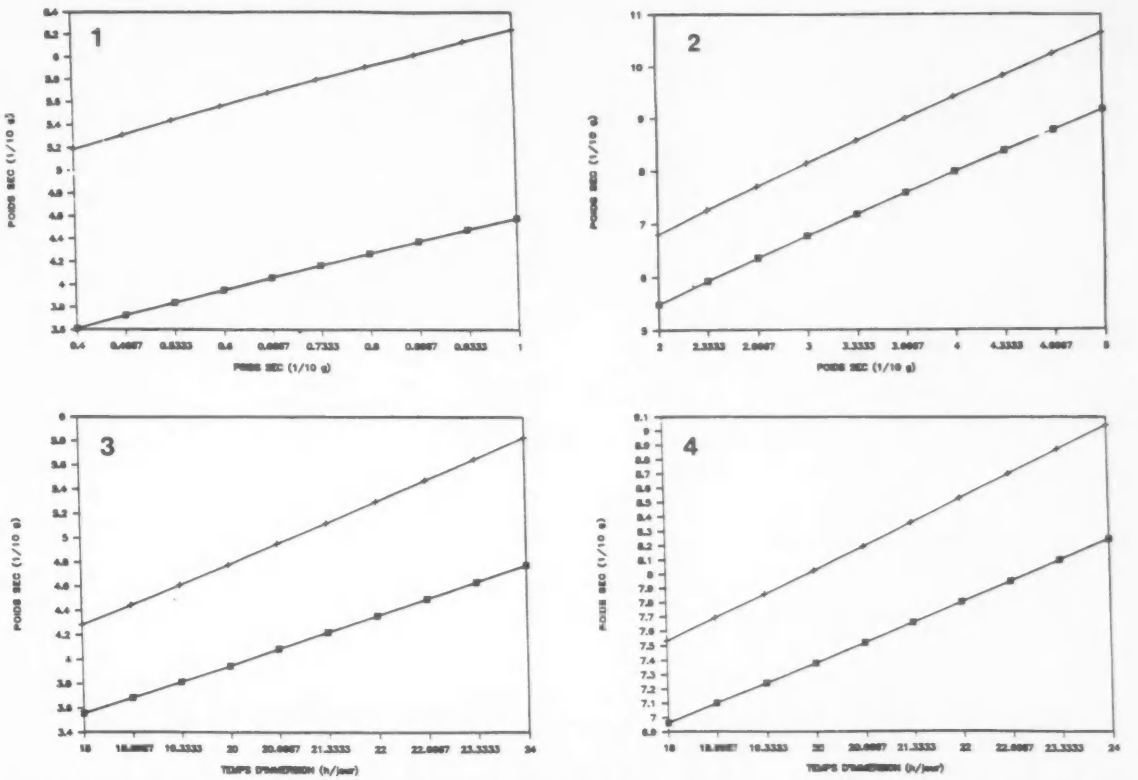

FIG. 6. Simulation de la croissance potentielle (1/10 g) sur les sites de Nole (□) et de Lilleau (+) : 1) en fonction du poids initial (1/10 g) pour les classes 1 (1) et 2 (2); 2) en fonction du temps d'immersion (h/d) pour les classes 1 (3) et 2 (4). Pour un même poids initial ou un même temps d'immersion, le site de Lilleau présente toujours une croissance supérieure.

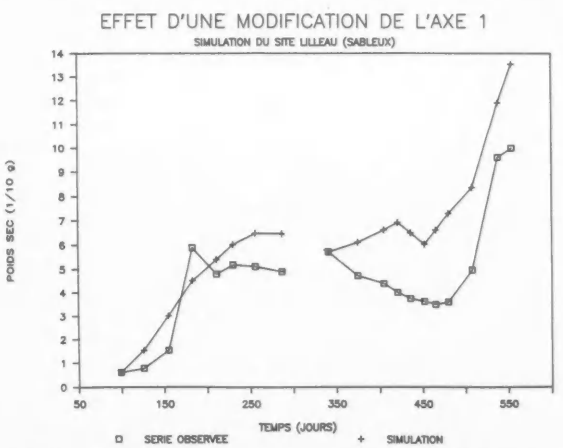

FIG. 7. Simulation de la croissance potentielle (1/10 g) du site de Lilleau avec modification de l'axe 1 (+) et observations (□). La différence de sensibilité des sites vaseux et sableux à l'axe1 (sédiment) s'explique par l'effet inhibiteur lié à la remise en suspension.

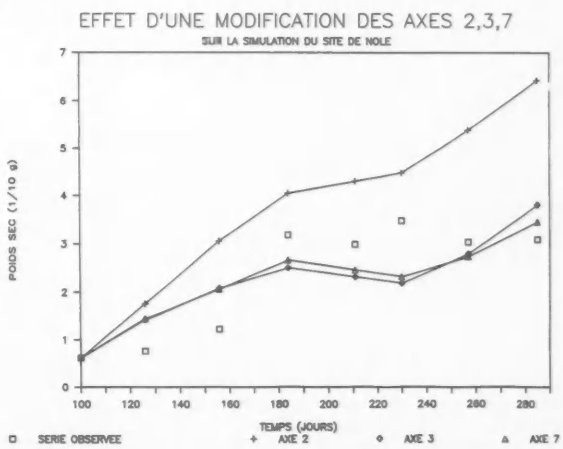

FIG. 8. Simulation de la croissance potentielle (1/10 g) du site de Nole avec modification de l'axe 2 (+), 3 (◇), 7 (△), du site de Lilleau et observations (□).

duction différents entre les sites, aucune information directe ne peut découler des observations. Les simulations à poids initial ou à temps d'immersion constant (fig. 6) conservent les mêmes tendances par rapport aux observations : les croissances des palourdes sur le site de Lilleau sont toujours supérieures.

Une simulation réalisée sur le site de Lilleau en utilisant les coordonnées des paramètres sédimentaires du site vaseux (axe 1) indépendamment des caractéristiques hydrologiques, tend à

montrer que le facteur «quantité de nourriture» du sédiment n'est pas seul à intervenir (fig. 7). En effet la croissance est fortement favorisée dans le cas présent, en opposition avec les observations du site de Nole. Par ailleurs, des simulations au cours desquelles les valeurs des axes, 2, 3 et 7 du site 2 ont été substituées à celles des mêmes axes du site 1 (successivement), pendant la première période de croissance (1985), ont été effectuées (fig. 8).

Discussion

Classification des descripteurs

Les corrélations observées entre les populations de bivalves, la colonne d'eau et l'interface eau–sédiment nécessitent d'être confirmées par des études pluri-annuelles afin de vérifier l'influence de chaque variable et d'éviter des corrélations accidentelles (Héral *et al.* 1984). Cette étude a permis, par l'utilisation du modèle de croissance, de mettre en évidence les principales variables environnementales agissant sur les fluctuations du poids sec de l'animal. Les périodes de croissance sont associées à de fortes températures, teneurs en chlorophylle, lipides et protéines dans l'eau, alors que les périodes d'amaigrissement sont observées à faible température, forte turbidité et une teneur en nourriture détritique élevée. L'effet de la température a été démontré par de nombreux auteurs, en particulier sur *Ruditapes philippinarum* par Mann et Glomb (1978) et Mann (1979). Les taux de filtration et de respiration sont modifiés par la température selon Bernard (1983) et Laing *et al.* (1987). Par ailleurs, le contenu lipidique de la nourriture phytoplanctonique influence la croissance de la palourde japonaise selon Laing *et al.* (1987). Si les principaux descripteurs sont identiques au cours des deux années sur le site, la sensibilité dépend du niveau trophique (floraison phytoplanctonique). Par ailleurs, les conditions hivernales en classe 2, marquées par des remises en suspension de sédiment plus fréquentes, peuvent expliquer les différences de sensibilité.

Effet du site

L'effet du site est visualisé par les fortes différences de croissance en observation et simulations (fig. 4). Les palourdes localisées dans le nord du bassin présentent une croissance systématiquement plus élevée que la population située dans la partie médiane du bassin. Cet effet peut être dû : 1) à la différence dans le temps d'immersion moyen correspondant à un temps de filtration potentiel, 2) à la quantité de nourriture disponible dans la colonne d'eau et le sédiment, et 3) à la nature physique du sédiment, associée à la vitesse du courant.

Le temps d'immersion est considéré comme un facteur important en zone intertidale dans le contrôle de la croissance comme l'ont démontré Glock et Chew (1979) sur *R. philippinarum*, Griffiths (1981) sur *Choromytilus meridionalis*, Gillmor (1982) sur différents bivalves, et Goulletquer et Bacher (1989) sur *R. philippinarum*. Hormis le temps de filtration, l'émersion a des répercussions sur la physiologie de l'animal (Bayne *et al.* 1976 ; Fang 1982). Les simulations effectuées à temps d'immersion constants n'inversent pas la tendance mais diminuent les écarts observés entre les deux sites (fig. 6). Le temps d'immersion intervient par conséquent sur les performances finales de croissance sans être le principal facteur explicatif des différences observées.

Les déplacements des masses d'eau dans le bassin de Marennes-Oléron, ainsi que la biomasse en présence de cultures d'huîtres *C. gigas*, 82 000 t en 1985 (Bacher *et al.* 1986), interviennent sur la disponibilité en nourriture au niveau quantitatif et qualitatif entre la partie nord et médiane du bassin (Héral 1987 ; Bacher, com. pers.). Une déplétion de nourriture, occasionnée par l'activité de filtration des bivalves suspensivores est suggérée par certain auteurs : Fréchette et Bourget (1985a) montrent que la déplétion en matériel organique particulaire limite la croissance de *Mytilus edulis*. De même, Cloern (1982) et Cohen *et al.* (1984) suggèrent que les organismes benthiques sont présents en quantité suffisante pour réduire la biomasse phytoplanctonique dans les sites étudiés. Peterson et Black (1987) démontrent que la déplétion de nourriture dans la colonne d'eau constitue un effet prioritaire sur la croissance des suspensivores. Au cours de cette étude, la floraison phytoplanctonique automnale (axes 3–4, de l'ACP) est observée uniquement dans la partie nord du bassin. La biomasse phytoplanctonique se développant dans la partie médiane du bassin est de faible importance en raison d'une forte turbidité générale de la zone estuarienne (Héral *et al.* 1983). Du fait de la circulation générale nord–sud et du temps de résidence des eaux, il est probable que les floraisons phytoplanctoniques de moindre intensité sont consommées lors des passages successifs sur les élevages d'huîtres, avant d'atteindre le site de Nole. Par ailleurs, le contenu supérieur en matière organique au niveau du sédiment vaseux ne semble pas constituer un effet positif sur la croissance malgré la sensibilité des populations au descripteur 1 (fig. 5).

Selon Wildish et Kristmanson (1979), le couplage des facteurs vitesse du courant et rugosité du sédiment, régule la croissance des suspensivores avec une inhibition à des vitesses supérieures à 30 $cm \cdot s^{-1}$. La forte variabilité de la vitesse du courant sur le site de Nole, qui peut atteindre des valeurs instantanées supérieures à 1 $m \cdot s^{-1}$, faciliterait les remises en suspension ponctuelles. Si celles-ci sont facilitées par l'action hydrodynamique sur les sédiments vaseux par rapport au sédiment sableux (Jonge et van den Bergs 1987), la qualité nutritive de la nourriture (axe 2), définie par le rapport minéral–organique est affectée (Berg et Newell 1986 ; Bayne *et al.* 1987).

Par ailleurs Fréchette et Bourget (1985b) associent la matière organique particulaire remise en suspension près des zones d'élevage de *M. edulis*, principalement au phytoplancton filtré et rejeté sous forme de pseudofèces ou de fèces dues à une digestion partielle de qualité nutritive inférieure. Dans la partie médiane du bassin de Marennes-Oléron, Sornin *et al.* (1983) montrent également l'effet des biodépôts sur le contenu organique du sédiment ainsi que leur remise en suspension (Sornin *et al.* 1986). Ainsi à la différence de Baillie et Welsh (1980), la remise en suspension de l'interface eau–sédiment ne constitue pas nécessairement un avantage pour les filtreurs du fait de la qualité nutritive de la nourriture, en particulier de la surcharge minérale, qui peut occasionner des croissances négatives (Vahl 1980 ; Héral 1987). La simulation réalisée sur le site de Lilleau (fig. 7) tend à prouver que le seston minéral remis en suspension représente un facteur limitant de la croissance de *Ruditapes philippinarum* comme l'indiquent Daou et Goulletquer (1988). Toutefois, aucune corrélation significative entre le contenu minéral de l'eau et les paramètres du sédiment n'est observée (tableau 1). Ceci peut s'expliquer par la rapidité des processus au cours des remises en suspension (Jonge et van den Bergs 1987), phénomènes non décelables avec le pas de temps semi-mensuel adopté.

Sensibilité du modèle à la stratégie d'échantillonnage

Le modèle montre certaines lacunes dans l'explication de la

croissance sur les deux sites en 1985 (1re classe). Sur le site sableux une croissance très rapide est observée aux mois d'avril et de mai, suivie d'une ponte partielle non expliquée par le modèle. La croissance observée sur le site vaseux est plus régulière que la première. Le modèle a toutefois tendance à lisser la croissance en la surestimant légèrement au début du printemps, en l'affaiblissant de mai à juin pour l'accélérer considérablement en août.

Les différences obtenues en 1985 sont dues à une mauvaise représentation des relations trophiques ou bien du milieu. Ceci pose deux problèmes : celui de la formulation du modèle et de la limite de son pouvoir explicatif ou prédictif, et celui du suivi du milieu, c.-à-d. des variables choisies et du plan d'échantillonnage. La première catégorie de limitations de la méthodologie définie comprend l'existence possible d'effets seuils (création de pseudofèces par exemple), de déphasages (mécanismes de régulation de la filtration ou de l'assimilation) ou interactions non prises en compte. Dans la deuxième, en dehors des variables non suivies, un défaut d'explication peut provenir des biais d'échantillonnage ou du pas de temps retenu. La modélisation apparaît conforme aux observations en 1986. La méthode d'analyse a également été appliquée de façon satisfaisante en 1984 et 1985 (Goulletquer et Bacher 1989). On peut alors raisonnablement penser que le manque d'adéquation trouvé en 1985 vient d'un défaut du plan d'échantillonnage.

Des différences notables sont à rechercher dans les caractéristiques hydrologiques de chaque site. Les projections des observations sur les axes 2, 3 et 7 de l'ACP illustrent les particularités propres aux sites sableux et vaseux au cours de la première période de croissance (1985) (fig. 3). Le modèle est sensible principalement à ces 3 axes (68 % et 90 % de la sensibilité totale pour les sites 1 et 2 respectivement. Pour la période de début de croissance (mars à juin), on observe des différences systématiques entre les sites. La prise en compte de l'ordre de grandeur de la différence entre les projections et du coefficient de régression a_i de l'axe numéro i, incite à penser que l'axe 2 est responsable des différences de croissance ($\Delta \sim 2, a_2 = -3$). D'après les signes de Δ et a_i, on note que l'axe 2 a un effet inhibiteur sur la croissance sur site vaseux ($\Delta \cdot a_2 < 0$). Les modifications des axes 2, 3, 4 et 7 du site 1 confirment l'importance de l'axe 2 (fig. 8). Les résultats de l'ACP ont montré que l'axe 2 est lié positivement aux seston, protides, carbohydrates et température. Le retour aux données d'origine fait ressortir le lien entre les fortes valeurs des projections sur l'axe 2 et les valeurs, également fortes, du seston minéral pendant les mois de mai et de juin sur le site vaseux. L'importance prise ainsi par le seston minéral dans la sensibilité du modèle à des périodes cruciales de croissance et sur un site vaseux appelle des remarques sur la méthodologie employée. Il existe peut-être un biais des estimateurs de certaines variables hydrologiques. Une précision trop faible peut également générer des artefacts numériques dans le traitement non linéaire des données. Deux directions de recherche sont *a priori* envisageables : explication de la variabilité de certains paramètres hydrologiques et amélioration des estimateurs pour l'utilisation d'un pas de temps d'échantillonnage inférieur au mois.

Héral (1987) a déjà montré, sur un site vaseux, la corrélation existant entre la concentration particulaire et l'effet de la houle. Ward *et al.* (1984), Ward (1985) et Demers *et al.* (1987) ont mis en évidence l'effet du vent sur les concentrations particulaires dans des zones estuariennes de faible profondeur (entre 1 et 6 m). Force du vent, hauteur d'eau et rugosité du fond sont les paramètres contrôlant l'intensité de la remise en suspension. D'après ces mêmes auteurs, l'effet du vent est inférieur à celui du courant de marée jusqu'à un certain seuil de force du vent, au-delà duquel le premier devient prépondérant.

Dans une région à regime de veut irrégulier, une variabilité importante de certains paramètres hydrologiques peut donc se produire à une échelle de temps de l'ordre de quelques jours. Ainsi l'impact sur les mollusques ne peut être négligé à cette échelle, comme le suggèrent Fréchette et Bourget (1987), et impose une diminution du pas d'échantillonnage. Cette diminution aura pour conséquence d'améliorer le calcul de la réponse des mollusques aux variations du milieu. L'augmentation consécutive de l'effort d'échantillonnage sera compensée par un allégement en période d'amaigrissement hivernal, où les données de croissance sont relativement stables sur chacun des sites suivis. Enfin une loi empirique reliant la concentration particulaire à l'intensité du vent doit permettre de reconstituer le cycle du seston minéral à partir du régime de vent journalier, d'estimer et de corriger un biais du plan d'échantillonnage précédemment défini.

Remerciements

Les auteurs remercient M. P. Gros pour ses critiques, M. M. Héral et les arbitres anonymes pour leur aide constructive à la rédaction de la publication, ainsi que Mlle S. Taillade pour la frappe de ce manuscrit.

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Sigmoid Relationships between Nutrients and Chlorophyll among Lakes

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McCauley, E., J. A. Downing, and S. Watson. 1989. Sigmoid relationships between nutrients and chlorophyll among lakes. Can. J. Fish. Aquat. Sci. 46: 1171–1175.

Previous studies of freshwater eutrophication have shown that algal biomass tends to increase with the supply of dissolved phosphorus. This concept has been condensed into empirical relationships between chlorophyll a and total phosphorus concentrations (convenient measures of algal biomass and phosphorus availability) which have become essential tools in theoretical and applied limnology. With few exceptions, ecologists accept the idea that chlorophyll concentration rises linearly with phosphorus concentration among lakes. Such a suggestion runs counter to Liebigian principles of fertilization however, and contradicts laboratory and field research indicating the influence of other nutrients. Our analysis of two large independent phosphorus–chlorophyll data sets from temperate-zone lakes shows that log phosphorus–log chlorophyll relationships are sigmoid in shape and that a second nutrient, nitrogen, has a significant impact on chlorophyll concentrations when phosphorus availability is high. Our new empirical relationships indicate that mechanisms regulating algal biomass change with enrichment, and suggest new management strategies for polluted lakes.

Les résultats d'études antérieures sur l'eutrophisation des eaux douces montrent que la biomasse algale a tendance à augmenter avec l'accumulation de phosphore dissous. On a exprimé ce concept par des rapports empiriques entre la chlorophylle a et les concentrations de phosphore total (mesures pratiques de la biomasse algale et de la quantité de phosphore disponible), qui sont devenus des outils essentiels en limnologie théorique et appliquée. À de rares exceptions près, les écologistes admettent l'hypothèse selon laquelle la concentration de chlorophylle s'accroît de façon linéaire avec celle du phosphore dans les lacs. Une telle hypothèse est cependant contraire aux principes liebigiens de fertilisation et contredit les résultats de travaux en laboratoire et sur le terrain, qui font intervenir d'autres éléments nutritifs. Notre analyse de deux importantes séries de données indépendantes sur les rapports phosphore–chlorophylle dans les lacs de la zone tempérée montre que la relation entre la courbe logarithmique du phosphore et celle de la chlorophylle a une forme sigmoïde, et qu'un second élément nutritif, l'azote, a un impact significatif sur les concentrations de chlorophylle lorsque la quantité de phosphore disponible est élevée. Ces nouveaux rapports empiriques, qui indiquent que les mécanismes déterminants de la biomasse algale se modifient avec l'enrichissement, laissent entrevoir de nouvelles stratégies de gestion pour les lacs pollués.

Received August 26, 1988
Accepted February 23, 1989
(J9860)

Reçu le 26 août 1988
Accepté le 23 février 1989

Average phytoplankton biomass in lakes is strongly correlated with total phosphorus (TP) concentration (Sakamoto 1966; Dillon and Rigler 1974). This relationship forms the basis for lake management techniques (Dillon and Rigler 1975), and theoretical analyses (Smith 1982) of the dependence of algal growth rates on phosphorus availability. Estimates of TP have been used extensively by limnologists to assess the degree of eutrophication of particular lakes and to predict the productivity and biomass of virtually all biological components of lakes and ponds (Peters 1986). The fact that TP concentration in lake water is correlated with so many aspects of lake biology, along with experimental result (see below), have produced the concensus that phosphorus availability sets the limit to biotic productivity in temperate zone lakes (Hutchinson 1973; Schindler 1978; Peters 1986). While some have suggested that variables other than TP might significantly affect phytoplankton biomass (e.g. Smith 1979, 1982), there have been few detailed analyses of the shape of phosphorus–chlorophyll relationships.

The phosphorus–chlorophyll relationship presumably results from the dependence of algal growth rates on phosphorus availability. Extensive large- and small-scale experiments (Schindler 1977; Lean and Nalewajko 1979) show that phosphorus is the primary nutrient limiting algal productivity in most temperate zone lakes. The simplest nutrient-dependence hypothesis (Smith 1982) suggests that the response of algal biomass to changing phosphorus concentration might be linear. That is, a unit increase of phosphorus should yield an increment in plant biomass that is independent of the phosphorus concentration of the lake or pond. This might occur if all other plant nutrients are in infinite supply, and shifts in algal community structure or changes in the relative importance of decomposers or consumers do not systematically modify the response of plant biomass to phosphorus availability. An hypothesis that fits more closely with the classic Liebigian paradigm (von Liebig 1840; Hutchinson 1973; Droop 1974; Tilman 1982) would consider other nutrients that might limit algal growth at high levels of

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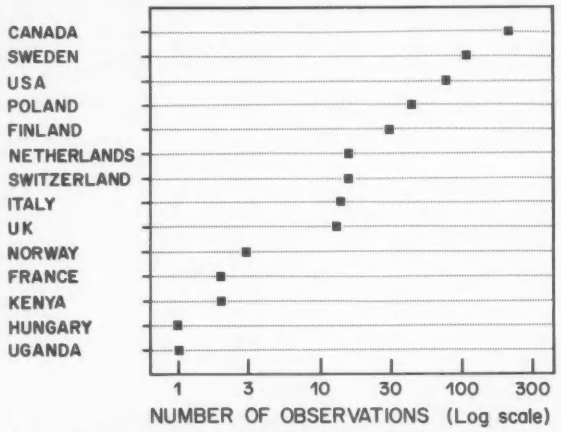

FIG. 1. Frequency of summer epilimnetic chlorophyll and summer epilimnetic total phosphorus (TP) data from 266 lakes in 14 countries, drawn from 67 published articles (reference list available on request from the authors).

ambient phosphorus. It would predict that chlorophyll should increase linearly until some other quantity (e.g. nitrogen, silicon, molybdenum, light) becomes limiting. A saturation point might thus be reached where subsequent enrichment with phosphorus would have little influence on chlorophyll concentration. Further nonlinearities might be seen if factors like competition of algae with bacteria for sparse phosphorus resources are important in lakes (Currie and Kalff 1984), or if herbivory changes systematically with enrichment (McCauley et al. 1988; Oksanen et al. 1981). These changes could alter the balance between algal growth and loss rates, and subsequently affect the equilibrium algal biomass. Relationships between TP and chlorophyll might therefore even be expected to be sigmoid in shape, and there is an ample theoretical basis to suggest that they could be nonlinear.

Despite the fact that nonlinear relationships between TP and chlorophyll among lakes are probable (Forsberg and Ryding 1980; Canfield 1983), there have been few quantitative examinations of their shape or form. A few studies (Sakamoto 1966; Dillon and Rigler 1974) have suggested that nitrogen availability modifies the chlorophyll yield per unit phosphorus, and that variation in the ratio of total nitrogen (TN) to TP could either help to explain "outliers"or reduce residual variability in phosphorus–chlorophyll relationships. Smith (1982) performed the most comprehensive analysis of phosphorus–chlorophyll relationships to date, and was the first to show that knowledge of both TP and TN concentrations could improve predictions of algal biomass. However, careful scrutiny of Smith's (1982) second figure reveals systematic lack of fit of a linear model to log TP:log chlorophyll data. This bias suggests that the fundamental relationship between TP and chlorophyll is, in fact, nonlinear. Straskraba (1985) has repeatedly argued, from a physiological basis, that the phosphorus–chlorophyll relationship is sigmoid. His limited analysis with a small data base (i.e. $n<30$) suggests that this is true at least within particular geographical regions.

Straskraba's (1985) critical analysis, reinforced by the systematic lack of fit of a linear model to Smith's (1982) data, led us to examine the form of the relationship between TP and chlorophyll *a*, and to test whether nonlinearities could simply be explained by the influence of nitrogen.

Data and Analyses

Limnologists now routinely measure chlorophyll *a*, TP, and TN concentrations, and analyses are fairly standardized: chlorophyll is measured by pigment extraction of filtered samples followed by spectrophotometry (Anon 1981); TP is determined by digestion of whole water samples followed by various modifications of the ammonium molybdate method (Murphy and Riley 1962); TN is measured as total Kjeldahl nitrogen (Anon 1981) of whole samples.

The results for a wide range of lakes are scattered throughout the ecological literature (Fig. 1). For example, the United States Environmental Protection Agency (USEPA) has single-handedly collected comparable data on 493 lakes (Taylor et al. 1978). We thus collected two independent sets of data: data drawn from the literature (Fig. 1; including the observations from Smith 1982), and data published by USEPA. We found a total of 548 published observations representing a range of TP from 1 to 14 093 $\mu g \cdot L^{-1}$ and a range of chlorophyll concentration from a 0.2 to 265 $\mu g \cdot L^{-1}$. Seventy percent of these observations were accompanied by summer epilimnetic total nitrogen (TN) measurements made at the same time (range of TN from 61 to 10 740 $\mu g \cdot L^{-1}$). USEPA (Taylor et al. 1978) data (493 lakes) included observations of summer epilimnetic chlorophyll, TP, and TN. USEPA data represented a range of chlorophyll concentration from 0.8 to 596 $\mu g \cdot L^{-1}$, TP from 5 to 4550 $\mu g \cdot L^{-1}$, and TN from 200 to 8204 $\mu g \cdot L^{-1}$. Thus, the combined data include 1041 measurements of average summer epilimnetic TP and chlorophyll *a* concentration (84% accompanied by TN measurements), with which to evaluate the form of the phosphorus–chlorophyll relationship and the influence of nitrogen. This represents an almost three-fold increase in sample size over the most ambitious published study (e.g. Smith 1982). Saline lakes (e.g. Prepas and Trew 1983) were not included in our data set.

The shape of the phosphorus–chlorophyll relationship was analysed in five steps. (1) Linear regression analysis was done on logarithmically transformed values of phosphorus and chlorophyll, as is usually done in the limnological literature. Transforming the observations acts to stabilize the variance. (2) Lack-of-fit in the log–log linear fit was examined by computing the residuals of this relationship, and determining their relationship to log TP using robust, locally-weighted sequential smoothing (LOWESS: Cleveland 1979). This weighted least squares technique aids the interpretation of the form of bivariate relationships, while guarding against the influence of deviant points. (3) The actual form of the relationship between log chlorophyll *a* concentration and log TP was then determined using robust locally-weighted regression analysis (Cleveland 1979). We did not apply model-based nonlinear regression analysis at this point because we wanted to avoid a priori judgements about the exact form of the functional relationship. Parameters used in all LOWESS analyses were delta = 0, $n-$steps = 2, and $f = 0.5$. (4) The relative roles of phosphorus and nitrogen in accounting for variation in chlorophyll concentration, and the possible role of nitrogen concentration in accounting for the nonlinearities in the log–log chlorophyll:phosphorus relationship were assessed using multiple regression analysis (Draper and Smith 1981), employing backward elimination variable selection (Hocking 1976). These multiple regression analyses were performed sep-

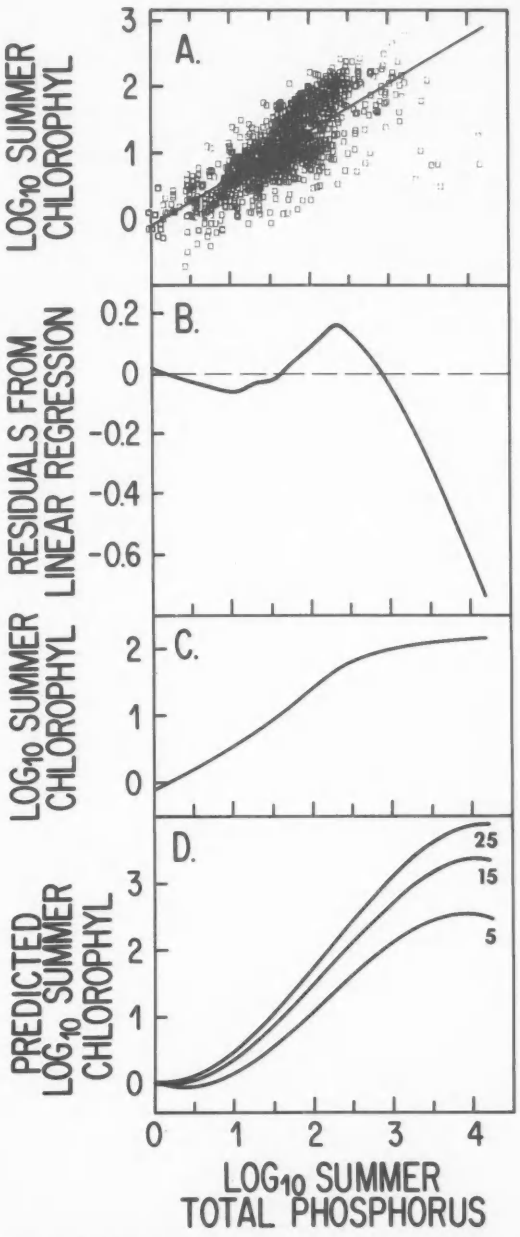

FIG. 2. Relationship between summer epilimnetic chlorophyll *a* concentration and summer epilimnetic total phosphorus (TP) concentrations in the world's lakes. (A) Combined observations from the data sources listed in the legend of Fig. 1. The line shows the linear regression obtained from a least squares analysis of the data. (B) The trend in residuals of a linear regression model fitted to the data in panel (A). Trends are estimated by locally weighted robust sequential smoothing (Cleveland 1979). (C) The overall trend in the relationship between chlorophyll and TP estimated by locally weighted robust sequential smoothing (Cleveland 1979). (D) Relationships between lake TP and chlorophyll concentrations predicted from regression models (combined model, data source-literature) presented in Table 1 for TN:TP ratios (by mass) of 5, 15, 25.

TABLE 1. Partial regression coefficients for effects of variables on $\log_{10}$ summer chlorophyll *a* concentration (micrograms per litre) found by least squares regression analysis. Variables were the logarithms of summer TP (log TP) and TN (log TN) concentrations (micrograms per litre), the square and the cube of the logarithms of these variables, the product of log TN and log TP, and, in the combined analysis of both data sets, a dummy variable signifying the source of the data (0 = published literature, 1 = USEPA). [a] indicates that the coefficient is not significantly ($p<0.01$) different form zero. The number of observations (n), coefficient of determination (r^2), and the overall F-value are presented for each analysis.

Variable	USEPA	Literature	Combined
log TP	−2.93	−0.77	−0.83
(log TP)^2	1.21	0.59	0.34
(log TP)^3	−0.24	−0.16	−0.12
(log TP) · (log TN)	0.45	0.31	0.43
Data source	—	—	−0.08
Intercept	1.46	0.03[a]	0.03[a]
n	493	382	875
r^2	0.61	0.80	0.71
F (overall)	191	375	431

arately for each of the two independent data sets (literature and USEPA). Candidate variables in both analyses were the logarithms of summer TP (log TP) and TN (log TN) concentrations (micrograms per litre), the square and cube of the logarithms of these variables to account for possible nonlinearities in the response, and the product of log TP and log TN to seek possible interactions between phosphorus and nitrogen concentrations. Initially all candidate variables were entered in the regression analysis, then variables were eliminated stepwise, based on their partial F-significance, until only the variables remained that had a partial F-significance of $\alpha<0.01$. (5) Finally, a global multiple regression analysis was performed on the combined literature and USEPA data sets. The analysis was performed exactly as in step (4) above, except that an apparent systematic underestimation of chlorophyll concentration in the USEPA data necessitated the addition of a dummy variable (Gujarati 1978) signifying the source of the data (0 = published literature, 1 = USEPA). (A complete set of tabular data is available, at a nominal charge, from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Ontario.)

Results and Discussion

Contrary to several previous analyses (Sakamoto 1966; Dillon and Rigler 1974; Nicholls and Dillon 1978; Smith 1982), we found a nonlinear relationship between the logarithm of chlorophyll concentration and the logarithm of TP concentration (Fig. 2 a–c). Figure 2a shows the results of a linear fit to the log TP:log chlorophyll relationship. The inadequacy of this linear relationship is visually apparent: chlorophyll concentration is overestimated at low phosphorus levels (2–50 $\mu g \cdot L^{-1}$) and underestimated at higher phosphorus levels (50–1000 $\mu g \cdot L^{-1}$). Figure 2b illustrates the precise form of the lack-of-fit by examining the relationship between the residuals of the linear regression and the logarithm of TP using LOWESS procedures (Cleveland 1979). Finally, analysis of the data by locally-weighted sequential smoothing (Cleveland 1979) reveals that the form of the relationship between log chlorophyll and log TP approximates a sigmoid curve (Fig. 2c). It is important to note that this nonlinearity occurs in the log–log relationship

between chlorophyll and phosphorus (Fig. 2a,c). The increment in the logarithm of chlorophyll concentration per unit change in log TP appears to increase over the range of 1–50 $\mu g \cdot L^{-1}$ TP, and above approximately 50 $\mu g \cdot L^{-1}$ TP the increments decrease in magnitude. These results support Straskraba's contention that the phosphorus–chlorophyll relationship is sigmoidal.

Polynomial regression analysis of two independent data sets (Table 1) shows that both $(\log TP)^2$ and $(\log TP)^3$ have highly significant partial effects on the logarithm of chlorophyll concentration. The sign and significance of effects are the same in both data sets providing independent corroboration of a curvilinear relationship. Higher order terms of log TP failed to enter significantly into the multiple regression model. The curvilinearity is highly significant even when data with TP>1000 $\mu g \cdot L^{-1}$ are excluded from the analysis. In addition, we have found (S. Watson et al., unpubl. data) that the relationship between algal biomass, estimated by cell volume, and TP among lakes is also nonlinear using logarithmically transformed observations. Thus, the nonlinearity is not due to variation in chlorophyll per cell with enrichment.

Our analysis shows that nitrogen has an unexpected effect on the relationship between TP and chlorophyll concentrations (cf. Smith 1982). TN concentration only accounted for significant variability in chlorophyll concentration as an interaction term with TP (Table 1). This term accounted for significant ($p<<0.0001$) variability in chlorophyll concentration in both independent sets of data (Table 1). Simply put, the effect of nitrogen on the phosphorus–chlorophyll relationship is not "a family of parallel lines whose position is a function of the TN:TP ratio" (Smith 1982). In reality, the effect of nitrogen is much greater at high TP concentration than low (Fig. 2d). The so-called phosphorus–chlorophyll relationship is a family of sigmoid curves that converge at low phosphorus concentration. Our analysis shows that the correlation between TP and chlorophyll concentrations is little affected by TN at low TP concentrations, but that chlorophyll concentration rises sharply with TN at high TP concentrations. Apparently, chlorophyll concentrations in the world's lakes are influenced both by phosphorus and nitrogen, with the latter playing a more dramatic role in phosphorus-rich lakes (Ahlgren 1980). Our observations suggest that covariation among lakes in phosphorus and nitrogen concentrations might determine features of the plateau in chlorophyll concentrations in lakes with high phosphorus levels (J. A. Downing et al., unpubl. data).

These findings influence current concepts of lake management. It was formerly assumed that a decrease in phosphorus loading of any water body would decrease the TP concentration and thus bring about a proportionate decrease in chlorophyll concentration, thereby improving water clarity (Dillon and Rigler 1974, 1975; Nicholls and Dillon 1978). Figure 2 shows that the rate of change in chlorophyll concentration with change in TP concentration varies with both TP and TN. At low TP concentrations (e.g. <50 $\mu g \cdot L^{-1}$) lakes are particularly sensitive to phosphorus loading because increases in TP concentration tend to accelerate the rate of change in chlorophyll concentration per unit change in TP concentration. Variation in TN concentration in oligotrophic systems has little, if any, consequence to algal biomass. In hypereutrophic conditions (TP>1000 $\mu g \cdot L^{-1}$), lake management by phosphorus effluent abatement would have little or no effect on chlorophyll concentration (Fig. 2c), whereas changes in nitrogen loading might bring about order-of-magnitude variations in algal biomass (Fig. 2d; Table 1). This agrees with experimental evidence from diversion experiments (Ahlgren 1980). Such treatment would not be without its costs, however, as reductions in nitrogen loading, without a reduction in TP concentration would decrease TN:TP, potentially driving the algal community structure toward noxious blue-green algae (Smith 1986).

Our analysis raises questions concerning how processes that influence the average algal biomass present in lakes, might vary with enrichment of phosphorus and nitrogen. If the temporal average of summer chlorophyll concentrations reflects the equilibrium algal biomass, then the shape of the phosphorus–chlorophyll relationship (Fig. 2c) indicates that the balance between average growth and loss rates of algae must vary systematically with lake enrichment. For example, increments of algal biomass per unit change in log TP increase, over the range of 1–50 $\mu g \cdot L^{-1}$ TP, suggesting a nonlinear increase in the difference between growth and loss rates. This would occur if changes in growth rates accelerate with increased phosphorus, or changes in loss rates decelerate, or both. Accelerating algal growth rates could arise from changes in algal community structure to competitively superior species moving from ultra-oligotrophic to oligotrophic lakes, or from shifts in the competitive balance between phytoplankton and bacteria. Loss rates imposed by herbivores, such as zooplankton, might not increase linearly because of the progressive establishment of carnivore communities affecting herbivore abundance. Similarly, the tendency of phosphorus–chlorophyll relationships to reach a plateau (i.e. decreasing increments in algal biomass over the TP range of 50–1000 $\mu g \cdot L^{-1}$), the elevation of which is correlated with TN concentration, suggests that nitrogen or some other factor is modifying the relationship between algal growth or loss rates and phosphorus.

Existing experiments cannot yet differentiate among these hypotheses. The challenge for theoretical ecology is therefore to elucidate the biological mechanisms that change with enrichment to produce the nonlinear response. McCauley et al. (1988) have used equilibrium predictions from simple predator–prey models in an attempt to provide mechanistic explanations for variation in plankton densities with enrichment. Perhaps the next step in the analysis of the relationship between algal biomass and nutrients would be a more detailed description of whether the nonlinear changes in algal biomass with enrichment can be attributed to variation in certain taxonomic or functional groups of phytoplankton. These observations might help to identify more precisely the processes changing with enrichment.

Acknowledgments

We thank V. Smith for generously providing data from his analysis, and D. Currie, R. W. Davies, L. Harder, P. Legendre, and F. J. Wrona for critical comments. This research was supported by grants from Natural Sciences and Engineering Research Council to E. McCauley and J. A. Downing, from University of Calgary to E. McCauley and FCAR (Quebec) to J. A. Downing.

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Unifying Nutrient–Chlorophyll Relationships in Lakes[1]

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Prairie, Y. T., C. M. Duarte, and J. Kalff. 1989. Unifying nutrient–chlorophyll relationships in lakes. Can. J. Fish. Aquat. Sci. 46: 1176–1182.

We used data drawn from the literature to test some basic predictions derived from nutrient limitation theory. Contrary to expectation, nitrogen was not better correlated to chlorophyll in nitrogen limited lakes (as measured by the total nitrogen:total phosphorus (TN:TP) ratio). We also examined whether the variability in published relationships between chlorophyll *a* (Chla) and nutrient levels in lakes can be explained by differences in the relative concentrations of nitrogen and phosphorus (TN:TP ratio). Our results show that the coefficients and precision of the Chla = f(TP) and Chla = f(TN) regression equations vary systematically and concomitantly with TN:TP ratios, and are highest for lakes with TN:TP ratios of 23–28 (by weight). These findings were confirmed with independent data and explain the variability of published Chla = f(TP) relationships. We propose a model that predicts the coefficients of Chla = f(TP) and Chla = f(TN) equations for lakes with different TN:TP ratios. This model proved useful in predicting the chlorophyll trajectories of lakes over time. The results imply an "optimal" TN:TP ratio beyond which the relative rates of increase in chlorophyll with increasing nutrient concentrations are reduced.

Nous nous sommes servis de données tirées des ouvrages scientifiques pour vérifier certaines prévisions de base découlant de la théorie de la limitation par les substances nutritives. Contrairement à ce qu'on s'attendait, la corrélation entre l'azote et la chlorophylle n'a pas été meilleure dans les lacs où l'azote est limitée (telle que mesurée par le rapport AT:PT). Nous avons également vérifié si la variabilité observée dans les relations connues entre la chlorophylle *a* (Chla) et la teneur en substances nutritives dans les lacs peut être expliquée par les différences notées dans les concentrations relatives de l'azote et du phosphore (rapport AT:PT). Nos résultats montrent que les coefficients et la précision des équations de régression Chla = f(PT) et Chla = f(AT) varient de façon systématique et en fonction des rapports AT:PT et sont les plus élevés dans le cas des lacs où les rapports AT:PT varient de 23 à 28 (par poids). Ces conclusions ont été confirmées avec des données indépendantes et elles expliquent la variabilité des relations connues Chla = f(PT). Nous proposons un modèle qui prédit les coefficients des équations Chla = f(PT) et Chla = f(AT) pour les lacs ayant des rapports AT:PT différents. Ce modèle s'est avéré utile pour prédire les variations de la chlorophylle dans les lacs avec le temps. Les résultats laissent entendre qu'il y a un rapport AT:PT "optimal" au-delà duquel les taux relatifs d'augmentation de la chlorophylle et des concentrations des substances nutritives sont réduits.

Received June 14, 1988
Accepted March 9, 1989
(J9774)

Reçu le 14 juin 1988
Accepté le 9 mars 1989

The dependence of phytoplankton biomass on phosphorus (Schindler 1977, 1978; Dillon and Rigler 1974) is remarkably general in lakes. Since Sakamoto (1966) first demonstrated the strong empirical links between phosphorus, nitrogen, and chlorophyll and its subsequent generalization by Dillon and Rigler (1974), over 60 chlorophyll:phosphorus relationships have been published (cf. Peters 1986). Although the utility of these relationships in the management of eutrophication is not in dispute (OECD 1982), they have contributed also to a better understanding of nutrient limitation in phytoplankton (see Reynolds 1984 for review). Given that most temperate lakes are believed to be phosphorus limited, the strong correlation between phosphorus and chlorophyll concentration provides a general corroboration of Liebig's Law of the minimum applied to lake phytoplankton. Nevertheless, the full potential of nutrient–chlorophyll relationships as tests of Liebig's theory has not been fully exploited. For instance, phosphorus is expected to be a relatively poor predictor of phytoplankton biomass in nitrogen limited lakes, and conversely, nitrogen a poor correlate in phosphorus limited lakes. These predictions from the law of the minimum have, as yet, not been tested systematically.

Another troubling aspect of chlorophyll–phosphorus relationships is the substantial variability of the regression equations within and among lakes (cf. Smith and Shapiro 1981). The regression coefficients are sufficiently variable that, depending on the particular equation used, prediction for a single lake can differ several fold. Such differences may reflect the influence of other physical, chemical and biological variables on the chlorophyll:phosphorus (Chl*a* = f(TP)) relationship (Straskraba 1980; Smith and Shapiro 1981; Prepas and Trew 1983; Canfield and Jones 1984; Pace 1984). However, multiple regression analyses have shown that these additional factors do not explain much of the residual uncertainty. At most, their

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effect is to slightly raise or lower the intercept of the Chl*a* = f(TP) equation without affecting the slope (e.g. Smith 1982; Hoyer and Jones 1983). Yet, slope coefficients vary among studies (e.g. Stockner and Shortreed 1985; Prepas and Trew 1983) and such differences are not due to stochastic variation alone (Smith and Shapiro 1981; Ferris and Tyler 1985), although differences among data sets in measurement error of the predictor variables may well contribute.

Straskraba (1980) has argued that the slope of Chl*a* = f(TP) relationships changed systematically depending on the TP concentration, and that it reached very low values at high concentrations. Since hypereutrophic lakes are often characterized by very low total nitrogen:total phosphorus (TN:TP) ratios (Allan 1980), his results suggest that the slope of Chl*a* = f(TP) relationships in lakes is likely to vary between nitrogen deficient (low TN:TP) and phosphorus deficient lakes (high TN:TP). Smith (1982) has shown already that chlorophyll concentrations were strongly associated with the concentrations of both phosphorus and nitrogen (and consequently the nitrogen:phosphorus (N:P) ratio). However, his important study did not address whether the variability in the mathematical coefficients of Chl*a* = f(TP) relationships can be related to the lakes TN:TP ratio.

In the present paper, we consider two main hypotheses. First, we test whether predictions from nutrient limitation theory pertaining to nutrient–chlorophyll relationships are upheld. More specifically, do the relative strengths of phosphorus and nitrogen as correlates of phytoplankton biomass vary between phosphorus and nitrogen limited lakes (as measured by the TN:TP ratio). Second, we examine the hypothesis that differences in the slopes and intercepts of published Chl*a* = f(TP) equations may be explained by variation in the TN:TP ratio of the lakes comprising them. Our emphasis will be on predicting changes in Chl*a* = f(nutrient) regression equations from the TN:TP ratio of epilimnetic waters. The analysis begins with a description of our data base and how it compares with other general Chl*a* = f(nutrient) relationships. We then test whether the TN:TP ratio affects the regression coefficients of chlorophyll = f(nutrient) equations and assess whether differences in published relationships can be explained by differences in TN:TP ratios. Finally, we validate our findings using independent data.

Methods

Data Base

Concurrent measurements of TN, TP, and Chl*a* concentrations were drawn for 133 lakes from the limnological literature. Since we were interested in the influence of the TN:TP ratio on Chl*a* = f(nutrient) relationships, we sought to assemble a data set comprising lakes spanning a wide range in these properties. This was achieved by including lakes from the American, Canadian, Alpine, and Nordic contributions to the OECD (Organization for Economic Cooperation and Development) project (Janus and Vollenweider 1981; Rast and Lee 1978; Ryding 1980; Fricker 1980). We also included the data of Jones and Bachmann (1978). Empirical analysis involving several data sets often carry an additional uncertainty component: lakes are located in very different geographical and climatic regions and the sampling regime and intensity vary among studies. Although this reduces the precision of parameter estimates and therefore the power of statistical tests, we feel that the relationships that are revealed in such a data set are more robust and more general. The frequency distribution of TN:TP ratios in our lakes is shown in Fig. 1. A copy of the data set is available from the authors upon request.

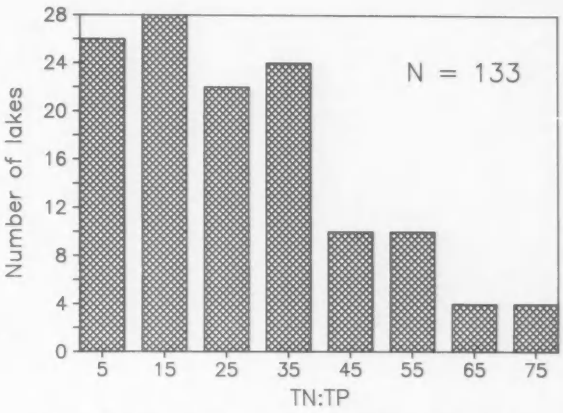

FIG. 1. Frequency histogram of TN:TP ratios in the data set.

Analytical Methods

Chlorophyll–nutrient relationships denoted throughout as Chl*a* = f(TP), Chl*a* = f(TN), and Chl*a* = f(TP,TN) refer to regression models after logarithmic (base 10) transformation of all variables. We used ordinary least squares for comparisons with literature equations or for the estimation of predictive models. When we sought only to identify the trend and shape of a relationship between two variables, we used the more flexible LOWESS technique (robust locally weighted regression: Cleveland 1979) implemented in PASCAL by A. Vaudor (Department of Biological Sciences, University of Montreal, Montreal, Que.). This smoothing method relies on the local fitting of polynomials to the data, using weighted regression analysis. The weights of data points are estimated iteratively using robust estimation techniques (Beaton and Tukey 1974). Rather than producing a mathematical equation, the LOWESS technique provides a description of the underlying shape of a relationship between two variables, without the usual constraints of traditional linear regression techniques.

Since most published Chl*a* = f(TP) relationships are constructed using lakes differing widely in their TN:TP ratios, it was not possible to use these relationships to examine our hypotheses directly. We therefore drew lakes with similar TN:TP ratios from our own data collection and ran regression analyses, and repeated the process for lakes with different TN:TP ratios. We automated this scheme on a computer using the following algorithm:

(1) All lakes were ordered by their TN:TP ratio. Starting at the lower end of the TN:TP spectrum and using a window width of one natural log unit,
(2) 15 lakes were randomly selected with replacement within the TN:TP ratio window (see Efron 1981),
(3) linear regressions between log-transformed values of TP, TN, and Chl*a* were performed, and regression statistics (correlation coefficient, intercept, slope, and mean TN:TP ratio) for the selected lakes were saved,
(4) the window was moved forward along the TN:TP spectrum by a distance of 0.25 natural log unit,

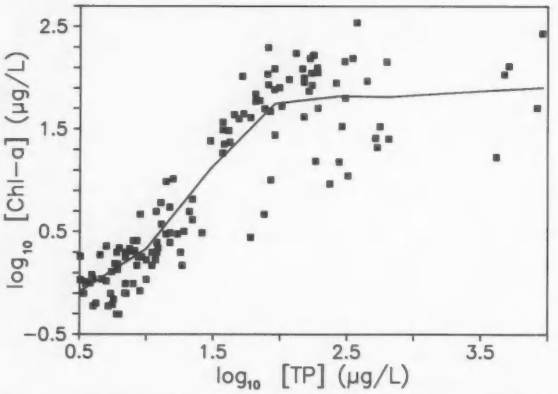

FIG. 2. Relationship between chlorophyll and TP concentrations (micrograms per litre) in 133 lakes. The solid line is the LOWESS fitted curve.

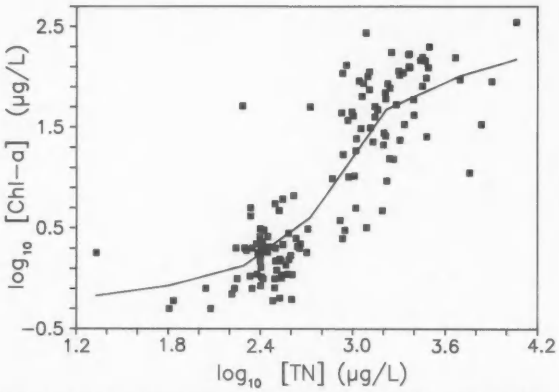

FIG. 3. Relationship between chlorophyll and TN concentrations (micrograms per litre) in 133 lakes. The solid line is the LOWESS fitted curve.

(5) steps 2, 3, and 4 were repeated until the complete spectrum had been covered.

Steps (2)–(3) were repeated five times per window. This process, together with the random selection with replacement, were used to adequately represent the stochastic variation among regression lines within a given TN:TP window (see Efron 1981). We chose the width of the moving window (one natural log unit) in order to ensure that the number of lakes was more or less equal among windows, and that a sufficient number (>10) of lakes were available for selection within each window. If there were fewer than 10 lakes in a given TN:TP window, no regression was performed. Since the whole spectrum of TN:TP ratio was covered with a total of 20 overlapping windows, a total of about 100 regression equations were calculated. Finally, the six resulting scatterplots (correlation coefficients, intercepts, and slopes as a function of TN:TP ratio for both the Chl$a$ = f(TP) and Chl$a$ = f(TN) regressions) were processed with the LOWESS technique in order to derive the underlying trends. We used the LOWESS parameter values ($\delta = 0.5$, $f = 2$) suggested in Cleveland (1979).

Results and Discussion

General Nutrient–Chlorophyll Relationships

Ordinary regression analysis of Chl$a$, TP, and TN data yielded the following equations:

$$(1)\quad \log_{10}\text{Chl}a = -0.390 + 0.874\log_{10}\text{TP} \qquad r^2 = .69$$

$$(2)\quad \log_{10}\text{Chl}a = -3.131 + 1.445\log_{10}\text{TN} \qquad r^2 = .69$$

$$(3)\quad \log_{10}\text{Chl}a = -2.213 + 0.517\log_{10}\text{TP} + 0.838\log_{10}\text{TN}\ r^2 = .81$$

All regression coefficients were significant ($p<0.0001$) and similar in magnitude to other published studies (e.g. Kerekes 1975; Canfield 1983). None of the regression coefficients of equation (3) differ significantly ($p>0.05$) from the corresponding equation of Smith (1982). Our results thus support his contention that both TP and TN are important variables in the prediction of chlorophyll *a* concentrations in lakes. Our data set differs from Smith's (1982) in that it covers a wider range of TP concentrations. Some of our lakes were extremely rich in phosphorus (>5000 μg/L), yet their chlorophyll levels were much lower than expected on the basis of the Chl$a$ = f(TP) trend for lakes with lower TP (Fig. 2). The chlorophyll concentration appears to reach a maximum beyond which further increase in phosphorus concentration does not result in higher chlorophyll concentrations. This asymptotic, or at least strongly curvilinear, nature of the general Chl$a$ = f(TP) relationship (Fig. 2) is not new to the literature (Forsberg and Ryding 1980; Straskraba 1980; Canfield 1983). The data of Schindler (1978); Smith (1982); and Vollenweider and Kerekes (1980) also exhibit a similar curvilinear trend. The LOWESS fitted line (solid line in Fig. 2) is nearly identical (once detransformed to arithmetic unit) to the logistic model of Straskraba (1980, his figure 3.31c). Our analysis and those of Canfield (1983) and Straskraba (1980) suggest that the "asymptote" is reached at about 100–125 μg/L TP.

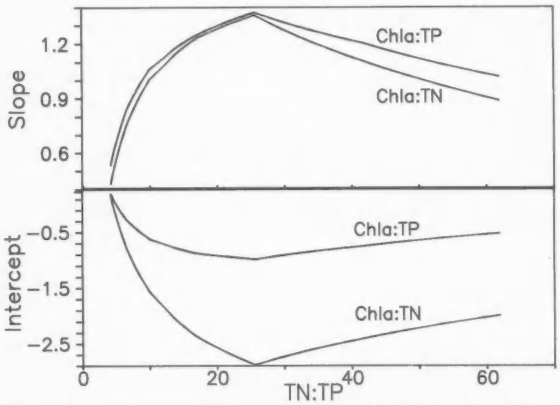

FIG. 4. Changes in the slope and intercept of Chl$a$ = f(TP) and Chl$a$ = f(TN) relationships with TN:TP ratio. The lines are LOWESS fitted curves to regression coefficients produced by the moving window algorithm (see text).

Although Chl$a$ = f(TN) relationships have not been investigated as extensively as Chl$a$ = f(TP), TN is as good or nearly as good a correlate of chlorophyll as TP in many cases (Forsberg and Ryding 1980) and better in some (Canfield 1983). For our data, TN and TP have the same predictive power but the slope is substantially steeper for TN than for TP (equations (1) and (2)). A LOWESS fitted line to our Chl$a$ = f(TN) data

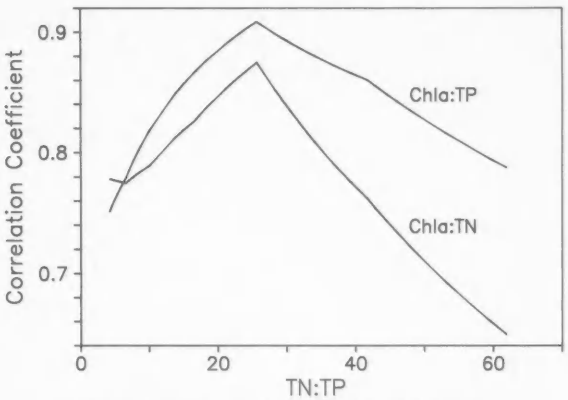

FIG. 5. Same as Fig. 4 for correlation coefficients.

suggest that the relationship is better described by a sigmoidal curve (Fig. 3) rather than by a straight line (Sakamoto 1966; Canfield 1983). Within the range of TN values observed in our lakes, no asymptotic value for chlorphyll can be derived on the basis of nitrogen; a conclusion that can be reached also from the scatterplots of Canfield (1983) and Forsberg and Ryding (1980). That the Chl*a* = f(TP) relationship exhibits a plateau, but that Chl*a* = f(TN) does not, cast some doubt upon the general belief that light limitation is responsible for the asymptote in nutrient rich lakes (Fig. 2). If this was the case, both Chl*a* = f(TP) and Chl*a* = f(TN) relationships would exhibit a similar asymptote.

TN:TP Ratio Effects on the Regressions

Before examining the influence of TN:TP on Chl*a*:nutrient relationships, it will be useful to identify some other interesting relationships among nutrients. The logarithms of TP and TN are significantly correlated ($r = 0.71$). Similarly, the log(TN:TP) ratio is significantly related to lake trophy expressed as either log(TP) ($r = -0.80$) or log(chlorophyll) ($r = -0.47$) but the trend with log(TN) was not significant ($r = -0.15$; $p > 0.05$). Thus, in our data set, selecting lakes according to their TN:TP ratios also tends, albeit to a limited extent, to select lakes of similar phosphorus concentration.

Figure 4 shows the LOWESS fitted relationships between the slope and intercept coefficients of Chl*a* = f(TP) and Chl*a* = f(TN) equations and the TN:TP ratios of lakes as generated by the moving window algorithm. There is a clear region of TN:TP ratio (23–28) for which slopes of the Chl*a* = f(TP) and Chl*a* = f(TN) relationships are highest (about 1.35). At low (<15) and high (>35) TN:TP ratios, the increase in chlorophyll per unit increase in nutrient concentration is much lower. As a reminder, the slope of a log–log relationship characterizes the relative rate of increase between two variables, with the value 1 representing one-to-one proportionality (i.e. a doubling of *X* results in a doubling of *Y*). Thus, the highest slopes (>1) represent the greatest positive response of chlorophyll to changes in phosphorus concentration. The magnitude and the changes in slope coefficient with increasing TN:TP ratio are virtually identical whether we use TP or TN as predictor variable (Fig. 4), despite the very different slopes when all the data are included (equations (1) and (2)). Similarly, the intercepts of the two relationships change systematically with TN:TP ratio (Fig. 4). However, their absolute magnitudes for the two nutrients differ markedly (Fig. 4), reflecting differences in their respective scale of measurement (Riggs 1963).

The strength of the correlation between chlorophyll and nutrients followed again the same trend, with highest values at TN:TP ratio of 26 (Fig. 5). The superiority of TP over TN as a predictor of chlorophyll grows wider in lakes with high TN:TP ratios. In lakes with TN:TP>50, TN explains less than half of the variance in chlorophyll concentrations (Fig. 5).

Figure 4 demonstrates that the slopes of Chl*a* = f(TP) and Chl*a* = f(TN) regression equations change systematically with TN:TP ratio. These results thus show that the constancy of slope implicit in all Chl*a* = f(TN,TP) models (Smith 1982; Canfield 1983) is inappropriate. Our analysis indicates that the intercept of the Chl*a* = f(TP) line will also change, as previous models have suggested (Smith 1982). However, the strong inverse relationship between slopes and intercepts ($r = -0.95$) renders such a comparison somewhat tenuous in our case. It is important to note that in view of the obvious nonlinearity among slopes, intercepts, and TN:TP ratios, it would be very difficult at best to develop a single multiple regression model that could account for such widely changing behavior of phytoplankton biomass and nutrient levels at different TN:TP ratios.

Biologically, regression slopes may be interpreted as a measure of the ability of the phytoplankton to respond to changes in nutrient concentration (Margalef 1983, p. 275). Similarly, the corresponding correlation coefficients can be viewed as a measure of the strength of the nutrient–phytoplankton coupling. Thus, one would anticipate that, in nitrogen deficient lakes (i.e. low TN:TP ratios), the slope and correlation coefficient of the Chl*a* = f(TN) relationship should be higher than that of Chl*a* = f(TP) (e.g. Cleasson and Forsberg 1980). Conversely, Chl*a* = f(TP) slopes should be relatively higher at high TN:TP values. However, Figs. 4 and 5 demonstrate that Chl*a* = f(TP) and Chl*a* = f(TN) slopes and correlation coefficients vary concomitantly with increasing TN:TP. The magnitude of the slopes is very similar for both nutrients over the complete TN:TP spectrum. Furthermore, TP is consistently a better predictor of chlorophyll than TN (Fig. 5), with the possible exception of lakes with very low TN:TP ratios. At face value, nutrient limitation theory is inconsistent with the general empirical patterns relating nutrients and phytoplankton in nitrogen versus phosphorus limited lakes. However, the discrepancy may be resolved by distinguishing nutrient limitation of individual algal species from multispecies assemblages. Rhee (1978) proposed such a distinction based on the variability of optimal N:P ratios for phytoplankton species (e.g. Rhee and Gotham 1980). Although some evidence suggests that ambient TN:TP levels tend to select for community species composition with similar optimal N:P ratios (see Harris 1986, p. 153), the large number of species in lakes (cf. Kalff and Knoechel 1978) assures a variability around the mean optimal TN:TP of the assemblage, such that nitrogen and phosphorus limited species will both occur simultaneously (Suttle and Harrison 1988). Thus, comparable biomass increases may be expected from either phosphorus or nitrogen concentration changes, although the species composition is likely to differ depending upon the direction of the shift in the TN:TP ratio. Also supporting this view is that the TN:TP ratio, beyond which the relative rates of increase in chlorophyll with increasing nutrient concentration (both phosphorus and nitrogen) are reduced (23–28 by weight), corresponds closely to the average minimum N:P cell quotas (30 by weight) of several algal species as derived by Shuter (1978).

TABLE 1. Comparisons of predicted regression equations with those derived from the data in Smith (1982). Notice the similarity in slope coefficients of Chl*a* = f(TP) and Chl*a* = f(TN) relationships within each TN:TP ratio category.

	Chl*a* = f(TP)		Chl*a* = f(TN)	
	Intercept	Slope	Intercept	Slope
		TN:TP<10		
Obseved	−0.573	0.986	−1.627	1.072
Predicted	−0.271	0.837	−0.850	0.755
		10<TN:TP<20		
Observed	−0.725	1.117	−2.182	1.166
Predicted	−0.823	1.203	−2.159	1.150
		20<TN:TP<30		
Observed	−0.969	1.351	−2.888	1.371
Predicted	−0.973	1.358	−2.840	1.343
		30<TN:TP<45		
Observed	−0.680	1.239	−2.670	1.262
Predicted	−0.834	1.251	−2.490	1.190
		TN:TP>45		
Observed	−0.339	0.961	−2.106	0.967
Predicted	−0.450	0.952	−1.850	0.912

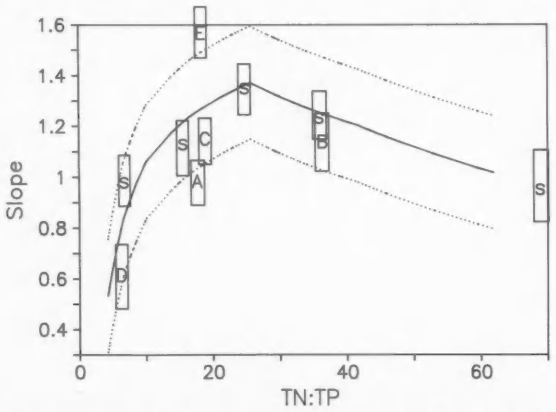

FIG. 6. Comparison of predicted and observed slopes of Chl*a* = f(TP) relationships of various studies in which lakes had a narrow range of TN:TP ratios. Broken lines are 1 SE of predictions from the LOWESS fitted curve (solid line). Boxes are 1 SE of slope estimates. S's are data from Smith (1982; see text and Table 1). (A) White (1983); (B) Ferris and Tyler (1985); (C) Smith and Shapiro (1981; TN:TP>10); (D) Smith and Shapiro (1981; TN:TP<10); (E) Sakamoto (1966).

The low slopes at low TN:TP ratios support the notion that algae use nutrients at a reduced efficiency in eutrophic lakes (Harris et al. 1983; Harris 1984), since high chlorophyll values usually occur in lakes with low TN:TP ratios. The less pronounced reduction in slope at TN:TP greater than 35 is more difficult to explain. The optimal N:P ratios of many eukaryotic algae, which dominate these systems (Smith 1983), are close to 20 (Rhee and Gotham 1980) such that growth in conditions departing from this value may be reduced. Thus, the fraction of the phytoplankton community with optimal N:P ratio deviating from ambient levels increases with increasing ambient TN:TP ratio. This hypothesis, however, clearly needs further testing.

Tests and Applications

To confirm the generality of the results and also to ensure that our conclusions were not artifacts of our analytical methods

TABLE 2. Coefficients of Chl*a* = f(TP) relationships for lakes of different TN:TP ratios. The equation is: $\log_{10}$ [Chl*a*] = intercept + (slope)·$\log_{10}$ [TP]. All concentrations are in micrograms per litre.

TN:TP	Intercept	Slope
5	0.028	0.643
10	−0.620	1.058
15	−0.823	1.204
20	−0.910	1.302
25	−0.979	1.365
30	−0.912	1.318
40	−0.776	1.210
50	−0.645	1.110
60	−0.530	1.030

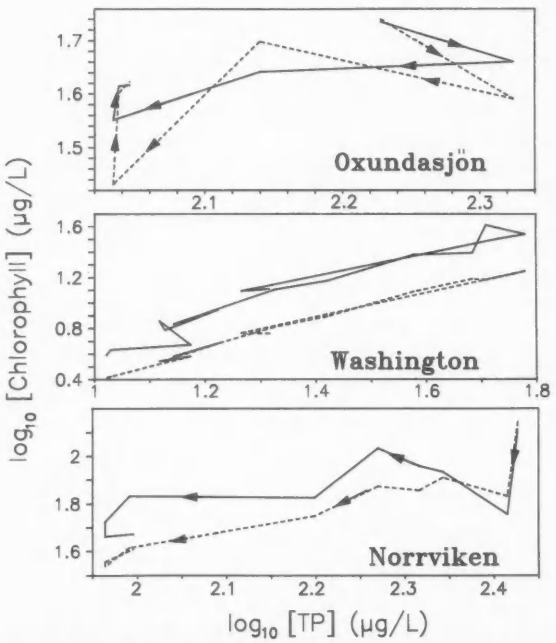

FIG. 7. Observed (solid line) and predicted (broken line) chlorophyll trajectories in (A) Lake Oxundasjön, (B) Lake Washington, and (C) Lake Norrviken. Arrows indicate time sequence.

(moving window algorithm and LOWESS fitting technique), we repeated the analysis with independent data and methods. To this end, we used the data compilation of Smith (1982). The data were divided into five TN:TP ratio categories (<10,10–20,20–30,30–45,>45) and separate Chl*a* = f(TP) and Chl*a* = f(TN) ordinary least squares regressions were run for each group. The results of the comparisons are shown in Table 1. As was the case for our data, the slope of the Chl*a* = f(TP) relationship changed substantially with TN:TP ratio, showing a maximum at TN:TP ratio between 20 and 30 (by weight). Also consistent with our analysis is the striking correspondence between the Chl*a* = f(TP) and Chl*a* = f(TN) slopes within each of the TN:TP ratio category.

We further tested whether our model (Fig. 4) could explain differences in regression coefficients of published relationships. To do this we compiled the few Chl*a* = f(TP) relationships in which lakes spanned only a narrow range of TN:TP ratios and compared the observed slope coefficients with those

predicted from Fig. 4 on the basis of the mean TN:TP ratio. Since none of these regressions covered lakes with low (<10) TN:TP ratios, we tested the adequacy of our model for that TN:TP range by constructing a Chl*a* = f(TP) regression with the lake-years with TN:TP<10 compiled in Smith and Shapiro (1981). The results for the published regressions showed a very good agreement with our model (Fig. 6) since none of the observed regression slopes were significantly different from the predicted ones. Our study therefore helps explain the variability in published relationships.

Utility of the Model for Prediction

Since TP provides better predictions more consistently (Fig. 5) for lakes of any given TN:TP ratio, Fig. 4 allows the calculation of the Chl*a* = f(TP) regression equation best suited to predict chlorophyll in any one lake. The precision of lake-specific regressions was compared with that of the best-fit simple (Chl*a* = f(TP) and multiple (Chl*a* = f(TP,TN)) regression models in our lakes. Using residual error variance estimates as the criterion of model precision, the results showed that our model reduces the error variance by more than 40% over the best Chl*a* = f(TP) model (equation (1)), and is thus significantly more precise ($F = 1.71$; $p<0.01$). Improvement over the multiple regression model (equation (3)) was only modest (7%) and not significant ($p>0.2$). However, the Chl*a* = f(TN,TP) model had a slight, but significant ($p<0.05$), tendency to underestimate Chl*a* at TN:TP and to overestimate it at high TN:TP values. No such bias was observed for our model. Thus, accounting for changes in slope of the Chl*a* = f(TP) relationship with changing TN:TP ratios definitely improves the predictive power of Chl*a* = f(TP) models and offers some gain in accuracy over Chl*a* = f(TN,TP) equations. The regression coefficients of Chl*a* = f(TP) for a given TN:TP ratio cannot be calculated analytically and must be estimated either graphically (from Fig. 4) or interpolated from a table of regression coefficients for the whole TN:TP ratio continuum (Table 2).

Predicting Temporal Chlorophyll Trajectories in Individual Lakes

The ability of a model to predict changes in chlorophyll within a single lake over many years is closer to the sort of utility lake managers seek in models (Lehman 1986). Since reduction in the phosphorus load to a lake is often accompanied by increasing TN:TP ratios (Smith and Shapiro 1981), we examined the adequacy of our model to predict the chlorophyll trajectory on a Chl*a* = f(TP) space for lakes experiencing changes in their nutrient concentrations. As test cases, we used two Swedish lakes (Lake Norrviken and Lake Oxundasjön; Ahlgren 1978) and Lake Washington (in Smith and Shapiro 1981). For each lake-year, the chlorophyll was predicted from the Chl*a* = f(TP) equation estimated on the basis of the TN:TP ratio for that year (Fig. 4). The results (Fig. 7) demonstrate a good agreement between the changes observed and those predicted from our model. This agreement is particularly striking in the case of eutrophic Lake Oxundasjön (Fig. 7a), with predicted and observed values differing by only 1–2 μg/L chlorophyll in 3 of the 6 yr. The predicted trajectory for Lake Washington was parallel to the observed one (Fig. 7b). However, our model consistently underestimated the observed chlorophyll levels by a factor of about two. This error compares favorably with the order-of-magnitude error inherent to most Chl*a* = f(TP) models (Dillon and Rigler 1974; Canfield 1983). The results from Lake Norrviken also confirmed the ability of our model to reproduce the chlorophyll trajectories in lakes with changing TN:TP ratios (Fig. 7c).

Conclusion

The TN:TP ratio affects the slope, intercept, and correlation coefficient of Chl*a* = f(TP) and Chl*a* = f(TN) relationships in lakes. However, both TN and TP correlated equally well with chlorophyll over the whole TN:TP range. This was contrary to expectations from nutrient limitation theory which predicted a tight coupling of phytoplankton with phosphorus in phosphorus-limited lakes (high TN:TP) and conversely with nitrogen in nitrogen-limited lakes (low TN:TP). The patterns were confirmed with independent data and also explained differences in slopes of other published relationships. This effect proved useful in predicting the temporal trajectories of chlorophyll in lakes of changing trophy. Chlorophyll = f(TP) relationships should thus be restricted to lakes with similar TN:TP ratios. Lakes with TN:TP ratios near 23–28 showed the steepest Chl*a* = f(TP) and Chl*a* = f(TN) slopes indicating a region where chlorophyll changes relative to nutrient concentration changes are largest. The next step towards a unification of eutrophication theory is to develop models predicting TN:TP ratio in different lakes.

Acknowledgements

This work was supported by scholarships from Les Fonds pour la formation des chercheurs et d'aide à la recherche and the World University Service of Canada to Y. T. Prairie and C. M. Duarte, respectively. We thank V. H. Smith, Department of Biology, University of North Carolina, for kindly making data available. D. Bird, R. Peters, K. Reckhow, and V. H. Smith made many valuable suggestions on the manuscript.

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Application of Statistical Power Analysis to the Oregon Coho Salmon (*Oncorhynchus kisutch*) Problem

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Nickelson (1986; Can. J. Fish. Aquat. Sci. 43: 527–535) was unable to reject the null hypothesis (H_o) of density-independent marine survival rate for Oregon coho salmon (*Oncorhynchus kisutch*) when wild, private hatchery, and public hatchery stocks were analyzed separately. Thus, even though there appears to have been no consistent increase in adult abundance in recent years in spite of large increases in smolt abundance, Nickelson's analysis does not support the alternative hypothesis (H_A) of density-dependent marine survival. Some fishery managers are using Nickelson's results to support proposals to increase smolt production further. I calculated statistical power for these cases, i.e. the probability that the null hypothesis of density-independence could have been rejected, even if marine survival were truly density-dependent. Power was below 0.19 for all cases, which meant that Nickelson (1986) had at least an 81% chance of making a Type II error (incorrectly accepting H_o), if H_o was actually false. Therefore, Oregon fishery managers should be cautious about making decisions on increased smolt production based on current data; they run a high risk of mistakenly assuming density-independent marine survival. More generally, managers should not take action based on a failure to reject a null hypothesis unless power is high.

Nickelson (1986; Can. J. Fish. Aquat. Sci. 43 : 527–535) a été incapable de rejeter l'hypothèse nulle (H_0) portant sur le taux de survie en milieu marin, indépendant de la densité, dans le cas du saumon coho de l'Orégon, lorsque les résultats obtenus avec des stocks en milieu naturel, une écloserie du secteur privé et une écloserie gouvernementale ont été analysés séparément. Ainsi, malgré l'absence apparente d'une augmentation régulière de l'abondance des adultes, ces dernières années, en dépit d'un accroissement important du nombre de smolts, l'analyse de Nickelson ne confirme pas l'hypothèse alternative (H_A) portant sur la survie en milieu marin en fonction de la densité. Certains gestionnaires des pêches utilisent actuellement les résultats de Nickelson pour justifier des projets d'accroissement des stocks de smolts. J'ai calculé la puissance pour ces cas, c'est-à-dire la probabilité que l'hypothèse nulle, qui comporte le concept d'indépendance vis-à-vis de la densité puisse être rejetée même si la survie en milieu marin dépendait vraiment de la densité. La puissance étant inférieure à 0,19 dans tous les cas, l'intervention de Nickelson (1986) présentait une probabilité d'erreur de type II (en considérant H_0 incorrectement), d'au moins 81 %, si H_0 était réellement fausse. En conséquence, les gestionnaires des pêches devraient être très prudents lorsqu'ils décident d'accroître les stocks de smolts en se basant sur les données actuelles : ils risquent fort d'admettre à tort une survie en milieu marin indépendante de la densité. Plus généralement, les gestionnaires ne devraient pas s'engager dans des projets lorsque l'hypothèse nulle ne'st pas rejetée à moins que la puissance ne soit élevée.

Received October 13, 1988
Accepted February 23, 1989
(J9908)

Reçu le 13 octobre 1988
Accepté le 23 février 1989

Hatcheries release large numbers of coho salmon (*Oncorhynchus kisutch*) smolts into the ocean in order to increase the number of adults caught by commercial and sport fisheries. This strategy appeared to work in the Oregon Production Area (northern California to southern Washington, U.S.A.) from the 1950s until about the early 1970s, after which there was a levelling off in mean adult abundance despite continued increases in smolt releases (ODFW 1982). This pattern continued until the early 1980s, when private hatchery operators began to reduce their releases of smolts (T. Nickelson, Oregon Department of Fish and Wildlife, Corvallis, OR, pers. comm.).

Researchers have debated these coho salmon data extensively. Some have found evidence of density-dependent marine survival (ODFW 1982; McCarl and Rettig 1983; Peterman and Routledge 1983; McGie 1984), while others have not (Peterman 1981; ODFW 1982; Clark and McCarl 1983). These differences in conclusions resulted from fitting different statistical models or from taking into account other factors such as oceanographic conditions. The most comprehensive analysis to date is Nickelson's (1986), which separately quantified smolt and adult abundances for wild, private hatchery, and public hatchery stocks. Nickelson (1986) tested the null hypothesis (H_o) that there was a linear relationship between smolt and adult abundances, which is equivalent to saying that the marine survival rate was density-independent. When each of these stocks was treated separately, he could not reject the null hypothesis.

Nickelson's (1986) analysis is statistically sound and he is properly cautious about the conclusions that can be drawn from his work. However, some fisheries managers in Oregon are using his results to justify considering further increases in hatchery production of Columbia River coho salmon smolts (T. Nickelson, pers. comm.). These managers are concluding that the failure to reject the null hypothesis means that marine sur-

vival really is density-independent, and further increases in smolt abundance are therefore justified.

The purpose of this paper is to use this example of Oregon coho salmon to illustrate the more general issue that this type of conclusion is not valid unless there is a small chance (β) of making a Type II error (i.e. accepting H_o when it is false). One should base important decisions on results of statistical tests that fail to reject a null hypothesis only if those tests have high statistical power ($1-\beta$), i.e. only if they have a low probability of incorrectly accepting H_o.

Statistical Power

Statistical power is an integral part of scientific hypothesis-testing, yet it has been largely ignored in the fisheries literature. By definition, statistical power is the probability of correctly detecting an effect, that is, rejecting the null hypothesis of no effect when in fact there really is an effect (Sokal and Rohlf 1969; Cohen 1977). Power is relevant to two situations important in fisheries management. First, experimenters should calculate how powerful some proposed experimental design is to determine the probability of detecting an effect, should one exist. Proposed experiments with low power can be modified (for example, by increasing sample size) to improve the chances of correctly detecting an effect. This is the most common application of the power concept in fisheries research. Second, power should be calculated and reported when a statistical analysis of some past data fails to reject H_o. Such a failure to reject H_o may occur because there really is no effect or because only a weak test of the hypothesis was possible; power will indicate the test's strength. Low power in this situation would mean that readers should put little credence in the conclusion that there is no effect, whereas high power would mean that a true effect is unlikely to exist. Statistical power is quite distinct from α, which is the main focus of most reporting of statistical analyses (α is the probability of making a Type I error, i.e. *rejecting* H_o when H_o is true). Scientists by convention are fairly conservative about this type of error and routinely set α at 0.05. β is the probability of making a Type II error, i.e. accepting H_o when H_o is not true. Despite its importance in some situations (such as testing the null hypothesis that some pollutant has no effect), the probability of Type II error has rarely been reported in the fisheries or other ecological literature. Some exceptions are Toft and Shea (1983); Peterman and Routledge (1983); de la Mare (1984); and Rotenberry and Wiens (1985). Power is simply $1-\beta$, and it therefore ranges between 0 and 1. Power can be calculated from standard equations and tables (Dixon and Massey 1969; Cohen 1977) and is a function of α, the sample size (N), and the effect size. Effect size is the magnitude of the true effect; the larger this is, the more likely a given experiment with a given sample size will correctly reject H_o at the stated α level.

Statistical power is directly relevant to the Oregon coho situation because in most of his stock cases, Nickelson (1986) failed to reject his stated H_o of density-independent marine survival. However, Nickelson (1986) does not state any power values for his analyses. Before making any further decisions, Oregon coho managers need to know how likely Nickelson (1986) was to reject H_o, if marine survival were actually density-dependent. I therefore calculated statistical power for his analyses and also performed a "reverse power analysis" to determine, for those cases in which H_o was accepted, how large the true degree of density-dependence would have to be to give a desired power.

Methods

Statistical Power

Nickelson (1986) assumed the following relationship between adult (A) and smolt (S) abundances:

$$(1)\quad A = cS^{\hat{k}},$$

where c and $\hat{k}$ are parameters. He fit the log transform of this relationship to his data by linear regression:

$$(2)\quad \log_e A = \log_e c + k \log_e S.$$

His null hypothesis was that $k=1$, in other words, that there is a linear relationship between A and S, which is equivalent to marine survival rates being density-independent. Nickelson (1986) used two-tailed tests and $\alpha=0.05$.

I calculated power for Nickelson's (1986, table 3) results using tables for the one-sample method for unknown standard deviation, σ (Dixon and Massey 1969, page 271). By convention, I calculated power for each of Nickelson's (1986) cases under the assumption that his estimated values, $\hat{k}$, were true. In other words, I calculated the probability that $H_o{:}k=1$ could have been rejected if the true k were equal to $\hat{k}$ in each case, using the standard error of $\hat{k}$, $SE_{\hat{k}}$, the sample size, and α, and assuming that $SE_{\hat{k}}$ is the same as SE_k. I also used a two-tailed test to be consistent with Nickelson (1986). In this a posteriori analysis, power is only relevant to the cases in which Nickelson (1986) did not reject H_o; I therefore omit discussion of the two cases of total populations in which he rejected H_o.

Users of power statistics usually want power to be at least 0.80 before they place credence in results that fail to reject a null hypothesis (Cohen 1977). However, users may differ in their desired levels of power. For example, with power = 0.95 ($\beta=0.05$) managers would be as cautious about Type II errors (incorrectly accepting H_o) as they normally are about Type I errors (incorrectly rejecting H_o).

Reverse Power Analysis

It is possible to obtain further information from a posteriori statistical power analysis. In the previous section, I assumed that $k=\hat{k}$ to estimate power, but if this were not the case, power values would differ. If managers can state the maximum probability of Type II error (β) that they are willing to tolerate, they can calculate through "reverse power analysis" (Rotenberry and Wiens 1985) the k value that would have to exist to give that β and associated power. To perform a reverse power analysis, they can use the four known entities, α, sample size, standard error of the parameter estimate, and the desired statistical power value (desired $1-\beta$), to solve for the remaining unknown, the effect size that must exist to give exactly that desired power. This procedure (Cohen 1977) is quite distinct from calculating a confidence interval on a parameter estimate, $\hat{k}$. In reverse power analysis, the value of the true k required to obtain the desired power will be larger or smaller than the 95% confidence interval, depending on the desired level of statistical power (see results below).

The reverse power analysis was performed by the following procedure. For a stated α, sample size, and a given power ($1-\beta$), I essentially worked backwards through a power table (Cohen 1977) but with these steps:

TABLE 1. Results of power and reverse power analyses for the groups of Oregon coho salmon and periods for which Nickelson (1986) was unable to reject the null hypothesis that $k = 1$. N = sample size in years, $\hat{k}$ and $SE_{\hat{k}}$ are the estimates of k and its standard error as reported in Nickelson (1986), and power = probability of correctly rejecting H_0 if $\hat{k}$ were true. Under "reverse power analysis," lower k and upper k are the values of k that would give power equal to the value at the top of the column.

					Reverse power analysis			
					With power = 0.95		With power = 0.80	
Test group	N	$\hat{k}$	$SE_{\hat{k}}$	Power	Lower k	Upper k	Lower k	Upper k
All years								
Wild	22	1.220	0.370	0.08	−0.41	2.41	−0.09	2.09
Public hatchery	22	1.049	0.290	0.04	−0.10	2.10	0.14	1.86
Private hatchery	6	1.086	0.074	0.15	0.66	1.34	0.74	1.26
Total hatchery	22	0.802	0.263	0.11	0.0	2.0	0.22	1.78
Strong upwelling years								
Wild	9	−0.69	1.376	0.19	−4.73	6.73	−3.46	5.46
Public hatchery	9	1.305	0.343	0.12	−0.43	2.43	−0.11	2.11
Total population	9	1.231	0.547	0.06	−1.28	3.28	−0.77	2.77
Weak upwelling years								
Wild	13	1.257	0.49	0.07	−0.94	2.94	−0.51	2.51
Public hatchery	13	0.993	0.202	0.03	0.20	1.80	0.38	1.62
Private hatchery	6	1.086	0.074	0.15	0.66	1.34	0.74	1.26
Total hatchery	13	0.861	0.183	0.10	0.28	1.72	0.44	1.56

1. A z score ($z_{1-\beta}$) was calculated for the desired power, using a standard normal distribution.
2. A z score ($z_{1-\alpha}$) was similarly calculated for the stated α.
3. The degrees of freedom, df, were $N-2$ because $\hat{k}$ was estimated from regression.
4. The parameter d, analogous to a t-statistic, was calculated from the equation (Dixon and Massey 1969, p. 273):

$$(3)\quad d = (z_{1-\beta} + z_{1-\alpha})\,(1 + (1.21\,(z_{1-\alpha} - 1.06))/df).$$

This equation closely approximated tabulated power values (<6% error overall, but <2% error for the range of values examined here).

5. The equation (Dixon and Massey 1969, p. 272):

$$(4)\quad d = (k - k_o) / SE_{\hat{k}},$$

in which d is analogous to a t-statistic, was rearranged to solve for $\hat{k}$,

$$(5)\quad k = d \cdot SE_{\hat{k}} + k_o,$$

where $\hat{k}_o$ was the null hypothesis value (= 1 here) and $SE_{\hat{k}}$ was the standard error of the estimated $\hat{k}$ value. Solving for $\hat{k}$ showed how large the deviation in true k would have to be to give the desired power. When d was positive (for $k > k_o$), equation (5) gave an upper k value that would meet that condition, and when d was negative, it gave a lower value. If the true k were between these upper and lower values, power would be less than desired, and β would be unacceptably high. These reverse power calculations were repeated for different levels of power.

Results

Statistical Power

All cases in which Nickelson (1986) failed to reject the null hypothesis of density-independent marine survival had low power (Table 1). In no case was there greater than a 19% chance of rejecting the null hypothesis of $k = 1$, if the $\hat{k}$ values were true. Thus, even if density-dependence existed to the degrees indicated by the $\hat{k}$ values in Table 1, Nickelson (1986) had a very small chance of rejecting H_o and thereby finding significant density-dependence. Expressed in terms of β, these power values mean that there was in every case at least an 81% chance of incorrectly accepting the H_o of density-independence.

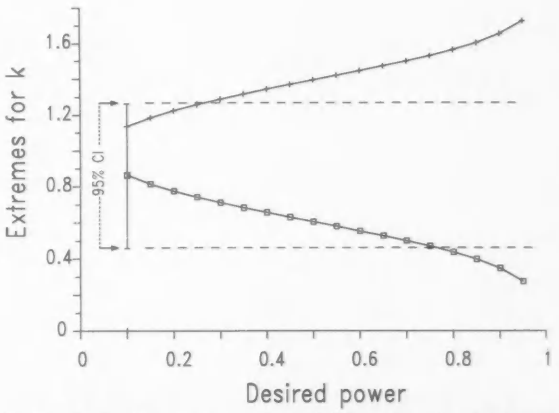

FIG. 1. Results of reverse power analysis for the case of total hatchery stocks in weak upwelling years. Curves show the upper and lower values of k that would have to be true in order to give the desired power. The 95% confidence interval (CI) around the point estimate of $\hat{k}$ is also shown for comparison.

Reverse Power Analysis

Reverse power analysis showed that in order for these cases to lead to acceptably low β (and acceptably high power), true k values would have to deviate much further from $k = 1$ than the current $\hat{k}$ values. For example, a power equal to 0.95 could only result if true $\hat{k}$ equalled either the lower or upper values shown under "power = 0.95" in Table 1. The case of total hatchery stocks in weak upwelling years (last row of Table 1) illustrates the interpretation of these reverse power values. In

that case, to obtain a power of at least 0.95, true k would have to be ≤ 0.28 (indicating compensatory mortality) or ≥ 1.72 (indicating facilitation or depensatory mortality). If this were not true, power would be below that desired and managers would not be justified in concluding that H_o was true; results would be equivocal. If managers were less restrictive and had desired power of only 0.8, true k values would not have to deviate as far from $k=1$ to lead to that power ("power = 0.8" column in Table 1).

These required extremes in k values vary continuously with desired power. Fig. 1 shows this for the N and SE_k given for the case of total hatchery fish in weak upwelling years. The higher desired power is, the further true k must be from $k=1$ to obtain that power. In contrast, note that the 95% confidence interval (CI) is independent of the desired power because the CI is determined by the data (Fig. 1).

Discussion

Statistical power is low for all of Nickelson's (1986) cases in which he failed to reject H_o because of the large year-to-year variability in the marine survival data, small samples, and possibly even errors in enumeration of abundances. Therefore, Nickelson (1986) had little chance of rejecting the null hypothesis, even if marine survival were actually density-dependent. Low power ($1-\beta$) means high β, or a high probability of making the mistake of accepting H_o when it is in fact false. Fishery managers are therefore not necessarily justified in using Nickelson's (1986) results to support a decision to increase abundance of coho smolts. Current data do not permit a strong test of the null hypothesis of density-independence and management actions should therefore not be based on them.

Reverse power analysis showed that in all cases, the parameter of density-dependence, k, would have to be well above or well below 1.0 in order to lead to acceptably high power (Fig. 1). This result also means that k could be almost that far from $k=1$ and still give unacceptable power. To take again the case in Table 1 of total hatchery stocks in weak upwelling years, if managers had a desired power of 0.95, a k as low as 0.27 (just below the 0.28 that would give power = 0.95) or as high as 1.71 would not give sufficient power (undesirably high β) and results would therefore be equivocal. However, few managers would consider a $k=0.27$ equivocal with respect to its implications; at that value of k, a doubling of annual smolt releases from 50 to 100 million at considerable cost would only lead to a 21% increase in mean adult abundance. In contrast, a $k=1.71$ would lead to recommendations for more hatcheries. This wide range in possible outcomes, and the lack of clear support from the data for any one of them, should make managers generally cautious about taking actions based on a failure to reject a null hypothesis without first estimating statistical power.

One way to increase power for the Oregon coho data is to increase the sample size by adding more years of information. However, when analyzing a fixed set of past data, managers are restricted to the methods described here.

Ironically, large increases in smolt abundance may be the best way to obtain significantly better $\hat{k}$ estimates (Peterman and Routledge 1983). Such increases are not justified by Nickelson's (1986) findings, but rather by the increased chances of distinguishing between the null case of a linear relationship between smolt and adult abundances and a nonlinear relationship (the two diverge more as smolt abundance increases) (Peterman and Routledge 1983). Ideally, such experiments would have to be carried out with river-specific catch allocation and escapement information to permit establishment of replicate and control stocks and varied starting times for experimental increases in abundance to avoid confounding with simultaneous environmental changes (Hurlbert 1984; Stewart-Oaten et al. 1986; Walters et al. 1988). These experiments would be justified only if they have acceptably high power and generate sufficient economic benefits (see Peterman and Routledge 1983).

While power analysis does not lead to any firm conclusion about the true value of $\hat{k}$ in nature, it does provide managers with more information on which to base decisions. This information complements that which is normally provided by standard statistical analyses that focus on reporting α values. It is particularly crucial that fishery managers recognize the value of power statistics because the interpretation of results where some null hypothesis is not rejected is strongly affected by power. As pointed out by Toft and Shea (1983), power should be given as much prominence as the reporting of α values in the analysis of past results.

Acknowledgments

I thank Tom Nickelson for providing the stimulus to write up this work. I am also grateful to Michael F. Lapointe and Michael J. Bradford for their programming assistance. All of these people, as well as Richard Lockhart, also provided very useful discussions. Jeremy Collie, Murdoch McAllister, Larry Dill, Michael Lapointe, Darren Gillis, Tom Nickelson, Bruce McCarl, and an anonymous reviewer made very helpful comments on the manuscript. This work was supported by a Natural Sciences and Engineering Council of Canada Operating Grant and a Simon Fraser University Special Research Projects Grant.

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Lethality of Low pH and Al to Early Life Stages of Six Fish Species Inhabiting PreCambrian Shield Waters in Ontario

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Holtze, K. E., and N. J. Hutchinson. 1989. Lethality of low pH and Al to early life stages of six fish species inhabiting PreCambrian Shield waters in Ontario. Can. J. Fish. Aquat. Sci. 46: 1188–1202.

Lethality of low pH and Al to egg and fry stages of common shiner (*Notropis cornutus*), white sucker (*Catostomus commersoni*), walleye (*Stizostedion vitreum*), lake whitefish (*Coregonus clupeaformis*), smallmouth bass (*Micropterus dolomieui*), and largemouth bass (*M. salmoides*) was determined in a series of laboratory tests in soft (Ca = 4.0 mg/L) water. Low pH was lethal to cleavage eggs in the first 4 d of exposure, to eyed eggs in the immediate prehatch period and to fry following their transition to branchial respiration. Early life stage response to Al was determined by their sensitivity to low pH. Al prolonged survival of cleavage eggs at pH = 4.2, was detrimental to eyed eggs and fry at pH 4.4–5.4 and was most lethal within 0.3 pH units of the pH which was lethal in the absence of Al. In situ distribution of four of the six species was adequately explained by lethality of low pH alone to cleavage eggs or fry. Sensitivity to low pH and Al produced estimates of pH >5.9 (common shiner), pH >5.4 (lake whitefish, white sucker, walleye), and pH >5.1 (smallmouth and largemouth bass) for survival of early life stages in acidified waters.

On a mesuré expérimentalement la mortalité de l'oeuf et de l'alevin du méné à nageoires rouges (*Notropis cornutus*), du meunier noir (*Catostomus comersoni*), du doré (*Stizostedion vitreum*), du corégone de lac (*Coregonus clupeaformis*) et des achigans (*Micropterus dolomieui* et *M. salmoides*) à l'acidité et à Al en eau douce (4,0 mg de Ca/L). L'acidité était mortelle aux oeufs en clivage durant les 4 premiers jours, aux oeufs oeillés durant la période précédant immédiatement l'éclosion et aux alevins après le passage à la respiration branchiale. La réaction à l'aluminium était conditionnée par la sensibilité à l'acidité. L'Al a prolongé la survie des oeufs en clivage à pH<4,2, mais il a nui aux oeufs oeilés et aux alevins à pH 4,4–5,4 et atteint sa létalité maximale à moins de 0,3 unités pH de l'acidité mortelle en l'absence d'Al. La distribution *in-situ* de quatre des six espèces s'explique assez bien par la létalité de l'acidité seule aux oeufs en clivage et aux alevins. À partir de nos résultats, on estime que les stades jeunes du méné peuvent survivre en milieu acide à pH>5,9, ceux du corégone, du meunier et du doré, à pH>5,4 et ceux des achigans, à pH>5,1.

Received May 18, 1988
Accepted February 10, 1989
(J9737)

Reçu le 18 mai 1988
Accepté le 10 février 1989

The impact of cultural acidification on fish populations has been the focus of extensive research, and considerable effort has been directed towards determining the extent of population losses (Magnuson et al. 1984; Wales and Beggs 1986; Schindler 1988) and the toxicological mechanisms behind them (Haines 1981; Spry et al. 1981; Witters and Vandenborght 1987). Research to date has identified H^+ and Al as the two most important toxicants and focused primarily on their toxicity to early life stages of fish (Muniz and Leivestad 1981; Baker and Schofield 1982; Witters and Vanderborght 1987). The emphasis on early life stage response can be justified because recruitment failure is a well documented signal of population stress in acidified waters (Harvey and Lee 1982; Mills et al. 1987) and because well defined relationships between early life stage and whole life cycle responses exist for a variety of pollutants (McKim 1985; Van Leeuwen et al. 1986).

Prediction and explanation of losses or sensitivity of individual species of fish on the basis of low pH and Al toxicity is confounded by several factors. Although Baker and Schofield (1982) demonstrate a dominant role of Al in oversaturated solutions at pH 5.2–6.4 recent studies (Campbell and Stokes 1985; Hutchinson et al. 1987) and proceedings of a symposium (Witters and Vanderborght 1987) conclude that the effects of pH on Al toxicity are not so straight forward. Documentation of pH/Al toxicity to fish is limited primarily to salmonid species (Witters and Vanderborght 1987). Testing of more sensitive species such as cyprinids may show different patterns of pH/Al interaction. In addition, species spawn at different times and places and early development rates vary. As a result patterns of chronic or episodic exposure differ among species. Prediction or description of losses of individual species may not, therefore, be based on laboratory estimates of survival of a single life stage.

A final problem confounding prediction is relating field surveys, which correlate fish presence to pH, with controlled laboratory studies demonstrating causation. Identical thresholds of species' responses in the field and in laboratory tests would provide very strong evidence that pH and Al were responsible for observed population losses. The sensitivities of individual species to low pH have been inferred from surveys and matched by the same relative sensitivity in laboratory testing (Rahel and Magnuson 1983). Fish in these tests, however, were exposed to steadily declining pH levels over the length of the experiment, lethal threshold effect levels were not established, Al

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TABLE 1. Summary of each test showing life history stage tested, duration of exposure in days, mean and range of exposure temperature (°C) and sample size as number of chambers (observations) × number of fish per chamber. Age of fish at start of each test is given in degree days (Σ days × °C) where data were available.

Species	Life history stage tested	Age	Duration of exposure	Mean exposure temperature and range	Sample size
Walleye	Cleavage egg through hatching	4	26	11.2 (9.5–12.5)	6×35
	Eyed egg through hatching	160	16	11.6 (10.5–12.5)	6×35
	Juvenile 0+	—	4	19.8 (19.0–20.5)	3×7
White sucker	Cleavage egg through hatching	4	18	13.0 (11.8–14.5)	6×35
	Eyed egg through hatching	134	8	13.8 (13.0–14.5)	6×35
	Yolk-sac fry	174	4	13.7 (13.0–14.0)	4×35
Common Shiner	Cleavage egg through hatching	11	14	13.3 (12.0–14.5)	4×35
	Eyed egg through hatching	54	9	13.7 (13.0–14.0)	4×35
Smallmouth bass	Eyed egg through hatching	≈ 54	11	18.0 (16.0–19.5)	6×25
	Swim-up fry	≈180	7	17.7 (16.0–18.5)	6×25
Largemouth bass	Juvenile (0^+)	—	7	21.0 (20.0–22.0)	4×9
Lake whitefish	Cleavage egg	91	12	6.5 (6.0–7.4)	6×25
	Eyed egg	371	12	6.5 (5.9–7.0)	4×15
	Swim-up fry	800	12	7.5 (6.6–8.6)	6×15

toxicity was not considered and only young-of-the-year fish were tested. The known influence of Al, life stage and exposure regime on fish response to low pH (Hutchinson et al. 1989) suggests that including those modifying factors in laboratory tests would improve the correspondence between laboratory and field estimates of pH sensitivity.

The purposes of our study were to: (1) verify the mechanism of population loss in acidified lakes, (2) resolve the influence of pH on Al toxicity and, (3) predict the impact of acidification on specific fish species by testing selected egg and fry stages of six species expected to show a wide variance in sensitivity to pH and Al.

The wide distribution of walleye (*Stizostedion vitreum vitreum*), lake whitefish (*Coregonus clupeaformis*), smallmouth bass (*Micropterus dolomieui*), and largemouth bass (*M. salmoides*) across acid sensitive waters (Wales and Beggs 1986; Neary and Dillon 1988) and their importance to the sport fishery of Ontario make documentation of their sensitivity to low pH and Al essential. The white sucker (*Catostomus commersoni*) and common shiner (*Notropis cornutus*) are also distributed across a wide range of acid sensitive waters and constitute an important forage base for sport fish and other components of the aquatic system (Scott and Crossman 1973). Accordingly, responses of egg and fry stages of these six species to low pH and Al were assessed in a series of continuous-flow toxicity tests using concentrations of toxicants and major ions which are typical of acidified PreCambrian Shield waters (LaZerte 1984; Neary and Dillon 1988).

Methods and Materials

Test Protocol

A series of 14 laboratory tests were conducted between April 1984 and March 1985 to assess the lethality of pH and Al to selected early life stages of the six fish species. The duration of each test varied with both life stage and species tested according to the timing of individual developmental stages (Table 1). Testing included both continuous exposure of successive developmental stages and discrete exposures of individual life stages, in order to assess responses to both chronic and acute exposure.

Testing began within 6 h of fertilization for cleavage eggs of lake whitefish, walleye, and white sucker and within 20 h of fertilization for common shiner. Cleavage eggs of largemouth and smallmouth bass were not tested. For eyed eggs, tests were started upon appearance of a pigmented eye spot on the embryo. Eyed eggs of largemouth bass were not tested. Tests of cleavage and eyed eggs were continued until 4 d after hatching first exceeded 50% in pH 6.0/0 Al controls in order to assess posthatch survival of yolk-sac fry. For smallmouth bass testing was extended to 7 d posthatch.

Exposures of both lake whitefish egg stages were terminated after 12 d because their maturation time was 4–6 mo (Cucin and Faber 1985). At this time acute mortality had ceased. The survival of eyed eggs held for an additional 9 d, for example, did not change between 12 and 21 d ($p>0.05$ data not shown). Survivors were transferred to untreated lake water (pH 6.8) and survival monitored until 7 d posthatch.

Exposures of fry and juvenile stages of all species were continued until lethal thresholds were attained and so varied from 4 to 12 d in length. Fry of white sucker began escaping from test chambers after 4 d and so testing was terminated then.

Survival and hatch were recorded daily in all tests. Mortalities were recorded when opaque areas covered >50% of the egg surface and following cessation of heartbeat or complete loss of pigmentation in fry.

Test Fish

Egg or fry stages of all fish species were collected from populations reproducing naturally in circumneutral soft water lakes in central Ontario (pH 6.3 to 7.0; Conductivity 30–69 μS/cm). With the exception of smallmouth bass, eggs and sperm were collected from 4–46 spawning adults, dry fertilized and hardened in the water from which adults were taken. Newly fertilized eggs (2–3 d old) and yolk-sac fry (≈10 d old) of smallmouth bass were collected directly from nests of wild fish. Young-of-the-year largemouth bass (1.1 ± 0.31 g) were seined from a moderately acidic lake (pH 5.6). Juvenile walleye (age $0+$, weight 2.2 ± 1.44 g) were obtained from a hatchery stock (White Pine Fish Culture Station, Ontario Ministry of Natural Resources) originating from Lake Skootamata (pH 6.9 Con-

TABLE 2. Chemical composition of St. Mary's Lake dilution water. Values given are mean ± 1 SD of 6–36 measurements made during the April 1984–March 1985 test period.

Major ions (mg/L)			Trace metals (μg/L)			Other parameters (mg/L)		
Ca	4.0	(0.3)	Al — Total	20.0	(1.0)	Total inflection point alkalinity	5.9	(0.4)
			— Inorganic monomeric	2.0	(0.4)			
Mg	1.4	(0.2)	Fe	227	(96)	Dissolved inorganic carbon	1.9	(0.1)
Na	2.2	(0.3)	Mn	58	(47)	Dissolved organic carbon	3.2	(0.4)
K	0.7	(0.1)	Cu	2.8	(1.8)	Colour (hazen units)	18.7	(3.2)
Cl	3.4	(0.8)	Zn	6.0	(3.5)	Conductivity (μS/cm)	46.7	(1.8)
F	38.0	(3.6)*	Pb	<3	(0)	Dissolved oxygen	9.7	(0.6)
SO_4	8.5	(0.8)	Ni	<1	(0)			

*μg/L.

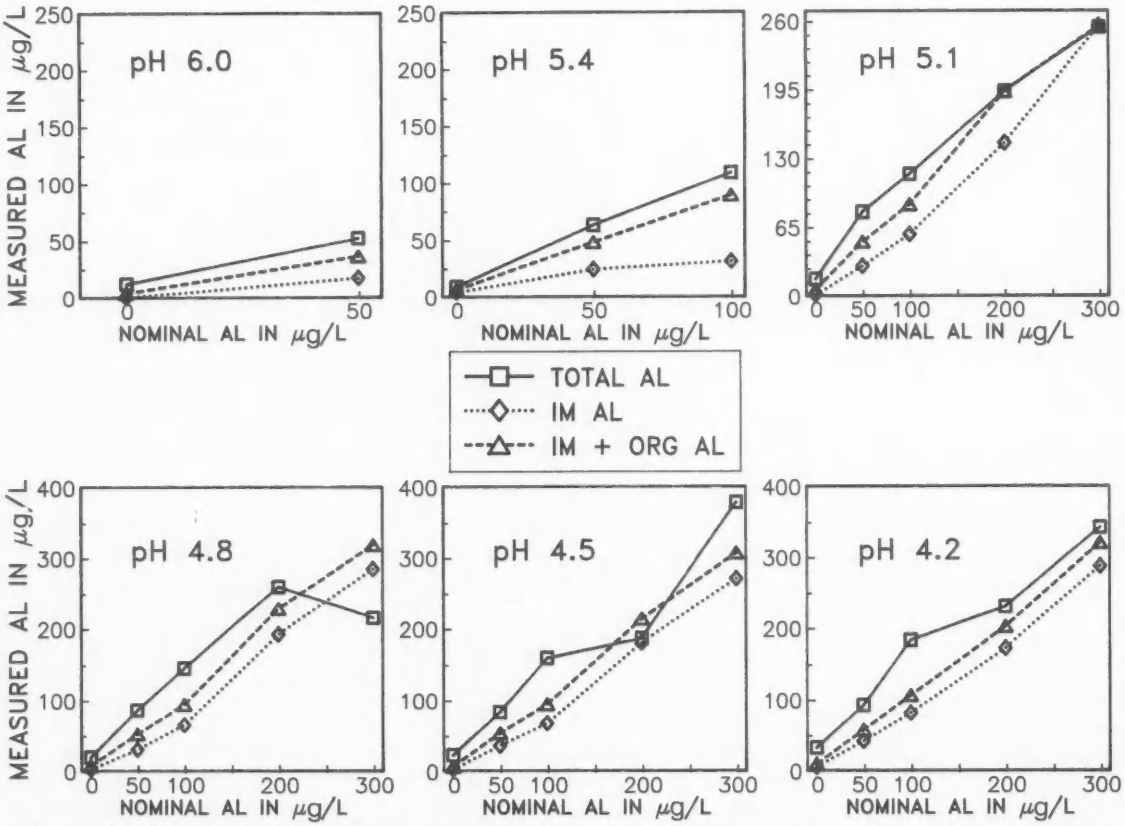

FIG. 1. Speciation of aluminum in test water. Measured concentrations of total Al, inorganic monomeric (IM) Al, and inorganic monomeric + organic (Org.) Al are plotted at each pH for different treatments of nominal total Al.

ductivity 49 μS/cm), and were reared from the swim-up stage in a hard water pond (pH 8.0; alkalinity 100 mg/L as $CaCO_3$). All eggs and fry were held in 6-L glass cylinders supplied with untreated dilution water (Table 2) prior to testing. Fish were fed brine shrimp nauplii during the holding period but not during the tests.

Eggs and fry were exposed in modified embryo-larval upwelling chambers (Holtze 1983) which used an air lift system to maintain toxicant flow through plastic chambers with nylon mesh sides. The numbers of eggs, fry, and chambers used varied between experiments and are summarized in Table 1. For juvenile fish, trays were removed and tanks divided into four

TABLE 3. Summary of pH, and total and inorganic monomeric Al measurements made over all tests. Values given are mean and SD of *n* measurements. pH and total Al are given for each treatment and inorganic monomeric Al is given for individual pH/Al combinations.

	Total Al (μg/L)				
Nominal	0	50	100	200	300
Measured mean	31	77	120	183	267
SD	(14)	(20)	(34)	(49)	(64)
n	371	334	311	256	231
pH	**Inorganic monomeric Al (μg/L)**				
5.97	0	17			
(0.13)	(0)	(2)			
305	50	11			
5.25	1	24	58	—	—
(0.22)	(1)	(4)	(13)		
351	41	41	42		
5.06	1	28	58	144	
(0.22)	(1)	(6)	(16)	(16)	
520	62	61	52	42	
4.79	2	30	65	192	284
(0.20)	(1)	(7)	(15)	(18)	(49)
667	62	64	62	52	52
4.60	2	40	70	119	288
(0.04)	(1)	(11)	(27)	(48)	(44)
185	21	21	21	22	10
4.50	2	35	67	180	270
(0.22)	(1)	(9)	(16)	(52)	(74)
481	53	53	51	54	54
4.39	13	51	76	146	228
(0.03)	(9)	(16)	(33)	(59)	(98)
185	20	20	20	21	22
4.22	5	41	81	172	287
(0.30)	(3)	(12)	(25)	(51)	(62)
663	62	63	63	65	62

chambers with perforated plastic panels.

The chambers provided physical separation of the four to six groups of fish used in each test but did not provide chemical separation as all chambers for a given treatment shared the same test water. Hence, fish in each chamber were treated as a single observation of population mortality for statistical analysis, as opposed to a true replicate.

Testing coincided with the natural reproductive cycle of each species and so water temperatures and photoperiod in each test were adjusted to match in situ levels. Lighting was provided from incandescent bulbs with staged on/off cycles to provide 1/2 h artificial dawn and dusk periods.

Exposure System

All tests were conducted in rectangular, 70-L polyethylene tanks containing 40 L of well aerated test water. Water used for holding fish and for toxicant dilution came from St. Mary's Lake (Ridout Twp., Ontario). Its chemical composition was similar to that of other low alkalinity waters in south-central Ontario except for slightly elevated levels of Na and Cl (Table 2) resulting from local road salting operations.

The ranges in pH (4.2–6.0) and total Al concentration (0–300 μg/L) selected for tests were similar to those reported in acidified, PreCambrian Shield lakes (LaZerte 1984; Neary and Dillon 1988). A total of 24 combinations of pH and Al were used for each test (Fig. 1). Concentrations of Al were added to achieve nominal levels of 0, 50, 100, 200, and 300 μg/L total Al at pH ≤4.8 but nominal levels were reduced to ≤200, ≤100, and ≤50 μg/L at pH 5.1, 5.4, and 6.0, respectively in an attempt to limit oversaturation and consequent formation and precipitation of polymeric and amorphous Al. Average measured levels of total Al (Table 3) did exceed theoretical solubilities of approximately 110, 33, and 9 μg/L at pH 5.1, 5.4, and 6.0, respectively (NRC 1986). Measured levels of inorganic monomeric Al (IMAl, Table 3) however, were at or below solubility and the difference between total Al and the IMAl fraction was accounted for by measured organic Al (Fig. 1) and analytical variability. This, plus the rapid replacement of test water (see below) minimized the formation and precipitation of any polynuclear hydroxy-Al species resulting from oversaturation (Hem 1968).

Stock solutions of acid and Al were prepared from reagent grade Al sulphate ($Al_2(SO_4)_3 \cdot 18H_2O$) or concentrated sulphuric acid (H_2SO_4) mixed with reverse-osmosis treated lake water. Acidity levels among Al stocks were equalized by adding 1.0N H_2SO_4. A pH stat system added acid to an aerated, constant level head tank for each pH treatment and pH adjusted water flowed to a 250-mL mixing chamber above each tank. Al was delivered to the mixing chamber at a constant rate of 2.3 ± 0.23 mL/min from a 250-mL constant-head reservoir. The combined flow of Al and pH controlled water from each mixing chamber was 225 ± 15 mL/min, and provided 90% molecular replacement of test solution to each tank in 7 h (Sprague 1973).

Water Chemistry

Samples were collected once weekly from the dilution water and at least two test tanks for determination of colour, conductivity, dissolved inorganic and organic carbon, alkalinity, major ions (Na, Ca, Mg, K, Cl, SO_4, F), and trace metals (Mn, Fe, Zn, Pb, Cu, Ni; Table 2). pH was measured one to two times daily in each tank. Mean values were within 0.0 to 0.15 units of nominal pH levels and were held to within 0.2 units over all tests. The single exception occurred during exposure of cleavage eggs of walleye when failure of the titration system allowed pH to rise to ≈pH 6.8. Nominal conditions were restored within 20 h. Total Al levels in each tank were measured daily by flameless AAS (atomic absorption spectrophotometry) on unfiltered samples. Al speciation was determined at least once weekly for each tank by the dialysis and oxine extraction method of LaZerte (1984), and was accompanied by measurement of dissolved inorganic carbon and free and total fluoride. All remaining analyses were carried out according to standard methods described by Locke and Scott (1986).

Mean, measured levels of pH, total Al, and IMAl varied between tests and between treatments within a test and so nominal levels of pH and total Al are used for clarity in all text and figures. Mean measured values for pH, total Al, and IMAl measured in each treatment are given in Table 3. Fig. 1 shows levels of organic, inorganic, and total Al measured in each treatment.

Statistical Treatment

Statistical analyses were initiated using an unbalanced, two-way analysis of variance (ANOVA) on arcsine-transformed observations of percent survival. Cell means from significant

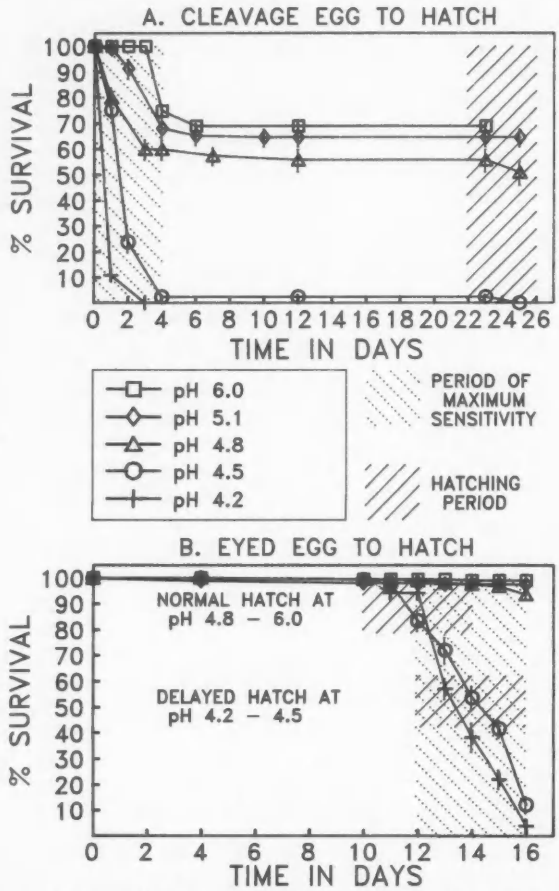

FIG. 2. Effect of low pH on survival of egg stages of walleye. Timing of lethality and hatching period are given for tests starting at fertilization (A, cleavage egg) and at the appearance of eyespot pigmentation (B, eyed egg). Error bars enclose 1 SD about the mean of six observations.

treatment effects were compared using Tukey's test and a probability level of $p<0.05$ was used throughout. Median lethal levels of pH (LC50s) were calculated using data on survival and mean measured pH for each Al treatment by the Spearman–Karber method (Hamilton et al. 1977). Cleavage egg mortality data were adjusted using Abbott's correction for control mortality prior to LC50 calculations as survival ranged from 51–95% in pH 6.0/0 Al controls. Mortality of cleavage egg controls likely reflected unfertilized eggs instead of system related stress as tests started at the eyed egg stage had <2% control mortality. Significant differences between LC50s were computed using the standard error of the difference (Sprague and Fogels 1977).

Results

Lethality of Low pH to Egg Stages

The time course of mortality varied substantially between cleavage and eyed egg stages. For all species tested, cleavage egg mortality occurred within the first 4 d of exposure and sensitivity changed little between then and 4 d posthatch (i.e. walleye, Fig. 2A). Thus median lethal concentrations (LC50s) for cleavage eggs were pH 4.62, 4.72, 4.86, and 5.42 after 4 d for lake whitefish, white sucker, walleye, and common shiner, respectively and did not change significantly over an additional 4–23 d of exposure (Table 4). At the onset of the eyed stage survival for the same four species was significantly reduced at pH 4.5, 5.1, 4.9, and 5.4 below that at control pH 6.0 (Table 5).

Survival of cleavage eggs in pH 6.0 controls ranged from 51–95.4% but two observations suggest that mortality was related to the presence of nonfertilized eggs and not to experimental procedures. First, cleavage eggs of common shiner, the most sensitive species tested, showed similar survival (≈50%) when held at pH 6.0 in the test and at pH 6.8 in untreated dilution water. Secondly, clearing in 10% acetic acid showed that 76% of the dead eggs from pH 6.8 had not been fertilized and so 76% of the common shiner control mortality of 49%, or 37.2% of the total mortality in cleavage egg tests resulted from nonfertilized eggs.

In contrast to cleavage eggs, eyed eggs of all species resisted low pH over most of their exposure period (i.e. walleye, Fig. 2B). With the exception of common shiner (LC50 pH ≈4.42), survival of eyed eggs of all species exceeded 50% at pH ≥4.2 during the first 4 d of exposure (Table 4).

Sensitivity of eyed eggs was greatest during the 4-d period immediately prior to and during hatch and was observed as both egg mortality and delayed hatch. Survival of walleye eggs at pH 4.2, for example, was reduced from 94.2% at 12 d to 3.9% by the time hatching was complete at 16 d. In contrast, survival of pH 6.0 controls for walleye was 99% when hatching ended after 14 d of exposure. Significant reductions in hatching success were observed at pH 4.8 for common shiner, and at pH 4.5 for walleye, white sucker, and smallmouth bass (Table 6). Hatching of common shiner, white sucker, and walleye was incomplete at pH ≤4.8 but, for white sucker, 61% of eggs hatched within 48 h of their transfer to untreated water. No hatching related sensitivity of lake whitefish was observed, as eggs were transferred to untreated water after 12 d. Survival exceeded 88% at pH ≥4.2 and few additional mortalities were recorded between the end of the exposure period and hatch (data not shown).

Lethality of Low pH to Fry Stages

Hatching of eggs did not change their sensitivity to low pH. Sensitivity of yolk sac-fry was established during the first 4 d, for tests started with cleavage eggs and in the 4 d prehatch period for tests started on eyed eggs. Mortality was <20% in the 4-d period following hatch for all tests (Tables 5 and 6) and LC50s increased by a maximum of 0.08 pH units after the first 4 d in cleavage egg tests or after hatch in eyed egg tests (Table 4). Survival of fry exposed from the eyed stage exceeded that of fry exposed from the cleavage stage in all tests (Tables 5 and 6). Significant reductions in 4-d survival of yolk-sac fry were recorded at pH 5.4, 4.9, 4.5, and 4.5 following exposure of cleavage eggs of common shiner, walleye, white sucker and lake whitefish, respectively (Table 5). Exposure of eyed eggs produced significant reductions in survival at pH 4.8, 4.9, 4.8, and 4.2 for the same four species, and at pH 4.5 for smallmouth bass (Table 6).

Yolk-sac fry of white sucker were extremely sensitive to low pH if exposures started after hatching. Although survival was 93.6% after 4 d of exposure to pH 4.8, all survivors were mor-

TABLE 4. Comparison of LC50 values for pH, with and without Al, determined in tests of continuous and discrete exposures of selected early life stages of six fish species. Confidence limits (95%) of LC50s are given in parentheses. Identical superscripts denote LC50 comparisons which were not significantly different ($p<0.05$).

Species	Total Al added (μg/L)	Cleavage egg			Eyed egg			Yolk-sac fry	Swim-up fry	Young-of-the-year
		4 d	Prehatch	4 d Posthatch	4 d	Prehatch	4 d Posthatch	4 d	4 d	4 d
Walleye	0	4.86^{KL} (4.82–4.91)	4.81^{KL} (4.78–4.85)	4.86^{LM} (4.82–4.90)	<4.2	4.62^{EF} (4.60–4.64)	4.70^{IJ} (4.66–4.74)	—	—	<4.51
	100	4.82^{JK} (4.77–4.87)	4.80^{K} (4.76–4.83)	5.28^{R} (5.24–5.31)	<4.2	4.65^{GH} (4.63–4.67)	5.05^{Q} (4.98–5.13)	—	—	<4.51
White Sucker	0	4.72^{J} (4.70–4.74)	4.71^{J} (4.69–4.74)	4.72^{J} (4.69–4.75)	<4.2	4.60^{EF} (4.58–4.61)	4.67^{HI} (4.65–4.70)	4.99*	—	—
	100	4.47^{D} (4.44–4.50)	5.01^{PQ} (4.98–5.04)	5.03^{Q} (5.00–5.07)	<4.2	4.69^{HIJ} (4.65–4.73)	5.02^{Q} (4.97–5.06)	>5.4*	—	—
Common Shiner	0	5.42^{S} (5.38–5.46)	5.46ST (5.41–5.51)	5.48^{T} (5.44–5.52)	4.42^{C} (4.39–4.45)	4.92^{NO} (4.89–4.94)	4.90^{MN} (4.87–4.93)	—	—	—
	100	5.41^{S} (5.35–5.46)	>5.29	>5.29	4.49^{D} (4.45–4.53)	>5.30	>5.30	—	—	—
Smallmouth Bass	0	—	—	—	<4.2	<4.2	<4.2	4.24^{A} (4.17–4.31)	4.98^{F} (4.69–4.99)	
	100	—	—	—	<4.2	<4.2	<4.2	<4.2	4.93^{D} (4.91–4.95)	
Lake Whitefish	0	4.62^{FG} (4.54–4.66)	—	—	<4.2	—	<4.2	—	4.38^{B} (4.36–4.41)	
	100	4.32^{B} (4.30–4.34)			<4.2	—	<4.2	—	4.56^{E} (4.52–4.60)	
Largemouth Bass	0	—	—	—	—	—	—	—	—	<4.2
	100	—	—	—	—	—	—	—	—	<4.2

*EC50 calculated for dead + moribund fry.

ibund and survival was only 5.7% at pH 4.5 (Table 7). An LC50 of pH 4.99 was calculated using dead and moribund fry (Table 4).

For smallmouth bass a large increase in sensitivity of sac fry was observed between 4 and 8 d of exposure, coincident with the transition from vitelline to branchial respiration. Survival at pH 4.2 was reduced from 92 to 0% of hatched fry during this period (Fig. 3A) and the LC50 increased from pH 4.24 (Table 4) to 4.43 (data not shown). Testing was repeated with posttransitional, 10–12 d old fry. Mortality was complete within 3, 24, and 72 h at pH 4.2, 4.5, and 4.8, respectively (Fig. 3B), and an LC50 of pH 4.98 was calculated (Table 4). Survival exceeded 95% at pH 5.1 (Table 7). The fry of smallmouth bass clearly passed through a period of high sensitivity to low pH following the transition to complete branchial respiration.

Fry tests for lake whitefish were started after swim-up and so mortality was related to pH and length of exposure rather than developmental stage (Fig. 4). The LC50 for swim-up fry increased from pH 4.38 at 4 d (Table 4) to pH 4.86 when lethal thresholds were attained at 12 d. Survival at pH 5.1 was 96.3% at this time (Table 7). This made swim-up fry of lake whitefish more sensitive than both egg stages, but egg exposures did not include the sensitive hatching period.

Young-of-the-year largemouth bass and walleye were tolerant of low pH. No mortality of largemouth bass fingerlings was observed during 7 d exposures to pH ≥4.2 and no other life stages were available for comparison. Walleye fingerlings were more tolerant than egg stages, but more sensitive than largemouth bass fingerlings. Lethal thresholds for walleye fingerlings were attained after 6 d of exposure, at which time survival exceeded 50% at pH 4.5, the lowest pH tested (Table 7).

Al Lethality

The response of early life stages to Al varied with the specific stage considered, length of exposure, pH, and concentration of Al. Overall, Al was beneficial to survival of cleavage eggs at lethal pH, detrimental to eyed eggs in the period immediately preceding hatch and detrimental to fry.

Al was beneficial to the survival of cleavage eggs during short periods of exposure at pH 4.2–4.5. For walleye, survival at 12 d ranged from 0% at pH 4.2 in the absence of Al to 26% at pH 4.2 + 300 μg/L of Al (Table 5). For white sucker survival at 6 d ranged from 1% at pH 4.5 to 74.6% at pH 4.5 + 300 μg/L Al (Table 5). Al only prolonged resistance time, however, as survival of both species was reduced to 0% by the time hatching was complete (i.e. walleye, Fig. 5A). Beneficial effects of Al on cleavage egg survival were also observed at pH ≤4.5 for lake whitefish, pH ≤5.1 for white sucker and at pH 5.1–5.4 (50 μg/L) for common shiner (Table 5).

Detrimental effects of Al on cleavage egg survival were observed at combinations of high pH and Al which suggested Al oversaturation. Survival was reduced at pH 5.4 + 100 μg/L and pH 5.1 + 200 μg/L Al for walleye, and at pH 6.0 + 100 μg/L Al for common shiner (Table 5). Intermediate levels of Al (50 μg/L) were beneficial to the survival of cleavage eggs of common shiner at pH 5.1 and pH 5.4 but higher levels (100 μg/L) reduced survival to that in 0 Al controls.

Al lethality to egg stages was observed in the immediate prehatch period and mortalities continued after hatching. Survival of cleavage eggs to 4 d posthatch was reduced at pH 4.8–5.4 with 100–200 μg/L of Al (Table 5), mostly as a result of posthatch mortality. Adding 100 μg/L Al, for example, increased

TABLE 5. Effect of pH and Al on survival of walleye, common shiner, and white sucker eggs exposed from the early cleavage stage to 4-d posthatch, and for lake whitefish cleavage eggs transferred to untreated water following 12 d of exposure. Length of exposure to each stage is given in days and age at beginning of each test is given in degree days. Identical superscripts denote no significant difference in means of four to six observations.

Total Al added (μg/L)	pH	Survival to eyed stage (12 d)					Fry survival to 4-d posthatch (26 d)				
		0	50	100	200	300	0	50	100	200	300
Walleye	6.0	68.9^{e}	62.1^{de}	—	—	—	67.9^{d}	56.8^{cd}	—	—	—
age 4°d		(2.6)	(6.8)				(2.6)	(6.0)			
	5.4	62.1^{de}	60.0^{de}	53.7^{cd}	—	—	61.1^{d}	60.0^{d}	42.8^{c}	—	—
		(7.3)	(4.6)	(8.7)			(7.4)	(4.6)	(13.8)		
	5.1	64.5^{de}	65.4^{de}	64.1^{de}	48.8^{c}	—	59.9^{d}	62.5^{d}	13.2^{b}	0.4^{a}	—
		(6.7)	(11.5)	(6.8)	(6.9)		(10.8)	(11.3)	(14.1)	(1.0)	
	4.9	55.6^{cd}	58.4^{cde}	57.0^{ce}	51.3^{cd}	51.7^{cd}	46.3^{c}	8.3^{b}	0^{a}	1.5^{a}	0^{a}
		(8.8)	(12.5)	(12.3)	(7.2)	(10.4)	(13.7)	(4.0)	(0)	(2.6)	(0)
	4.4	2.4^{a}	17.8^{b}	24.4^{b}	26.4^{b}	21.8^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
		(2.2)	(5.0)	(9.6)	(12.4)	(8.1)	(0)	(0)	(0)	(0)	(0)
	4.2	0^{a}	1.4^{a}	0^{a}	23.9^{b}	26.0^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
		(0)	(2.4)	(0)	(8.2)	(7.6)	(0)	(0)	(0)	(0)	(0)
				(4 d)					(14 d)		
Common	6.0	51.0^{f}	12.9^{c}	—	—	—	48.8^{e}	9.3^{b}	—	—	—
Shiner		(7.3)	(5.2)				(10.1)	(1.0)			
age 11°d	5.4	26.1^{d}	39.4^{e}	23.0^{d}	—	—	21.7^{c}	36.6^{d}	0^{a}	—	—
		(6.2)	(12.6)	(9.6)			(6.9)	(13.3)	(0)		
	5.1	1.4^{ab}	44.3^{ef}	4.1^{b}	0.7^{ab}	—	0^{a}	32.1^{d}	0^{a}	0^{a}	—
		(2.8)	(3.1)	(5.2)	(1.4)		(0)	(6.2)	(0)	(0)	
	4.8	0^{a}	2.9^{b}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
		(0)	(2.3)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
	4.5	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
	4.2	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
		(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
				(6 d)					(18 d)		
White	6.0	90.9^{fg}	89.9^{fg}	—	—	—	71.8^{fg}	72.3^{fg}	—	—	—
Sucker		(7.9)	(4.4)				(9.4)	(6.6)			
age 4°d	5.4	91.5^{fg}	89.4^{f}	90.7^{fg}	—	—	75.5^{g}	74.1^{g}	42.3^{c}	—	—
		(6.5)	(3.6)	(4.6)			(8.5)	(7.7)	(7.6)		
	5.1	80.1^{e}	90.4^{fg}	92.1^{fg}	98.0^{g}	—	60.2^{def}	76.5^{g}	50.1^{cd}	0^{a}	—
		(9.2)	(6.4)	(4.9)	(2.4)		(19.4)	(10.4)	(15.5)	(0)	
	4.8	72.4^{de}	87.5^{ef}	83.2^{ef}	76.6^{e}	61.5^{d}	64.7^{efg}	60.6^{de}	6.3^{b}	0^{a}	0^{a}
		(14.6)	(3.0)	(4.3)	(11.1)	(15.6)	(18.4)	(6.1)	(3.8)	(0)	(0)
	4.5	1.0^{a}	43.8^{c}	44.9^{c}	62.3^{d}	74.6^{de}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
		(1.5)	(12.7)	(12.1)	(11.8)	(5.6)	(0)	(0)	(0)	(0)	(0)
	4.2	0^{a}	0^{a}	2.8^{a}	18.5^{b}	31.8^{c}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
		(0)	(0)	(2.5)	(7.0)	(8.3)	(0)	(0)	(0)	(0)	(0)
		Survival to 12 d					Survival to 7-d posthatch				
Lake	6.0	95.4^{g}	90.0^{fg}	—	—	—	89.4^{fg}	82.0^{efg}	—	—	—
Whitefish		(4.5)	(8.3)				(3.2)	(13.3)			
age 91°d	5.4	92.1^{fg}	95.3^{g}	85.2^{efg}	—	—	69.8^{c-f}	93.3^{g}	56.0^{cd}	—	—
		(3.4)	(4.7)	(11.8)			(29.6)	(4.8)	(14.9)		
	5.1	90.6^{fg}	88.0^{fg}	89.5^{fg}	90.0^{fg}	—	85.3^{fg}	86.6^{fg}	75.5^{d-g}	62.0^{c-e}	—
		(7.7)	(5.4)	(6.1)	(5.5)		(6.3)	(5.4)	(19.9)	(31.1)	
	4.8	90.7^{fg}	96.0^{g}	88.0^{fg}	92.6^{g}	87.8^{fg}	86.0^{fg}	92.7^{g}	66.0^{c-e}	73.1^{def}	71.5^{df}
		(4.1)	(3.6)	(6.7)	(4.1)	(3.9)	(6.6)	(6.9)	(20.0)	(13.7)	(5.6)
	4.5	18.8^{c}	73.8^{e}	79.8^{ef}	88.0^{fg}	81.4^{ef}	14.1^{b}	70.4^{def}	53.5^{cd}	60.0^{cde}	51.0^{cd}
		(9.0)	(10.6)	(13.1)	(7.6)	(7.0)	(9.0)	(14.0)	(17.7)	(20.0)	(22.1)
	4.2	0^{a}	6.0^{b}	19.1^{c}	52.4^{d}	78.1^{e}	0^{a}	6.0^{ab}	16.5^{b}	44.4^{c}	59.5^{cde}
		(0)	(2.1)	(8.6)	(7.9)	(7.5)	(0)	(2.1)	(7.6)	(6.6)	(22.5)

TABLE 6. Effect of pH and Al on survival of walleye, common shiner, white sucker and smallmouth bass eggs exposed from the eyed stage to 4-d post-hatch. Length of exposure to each stage is given in days and age at beginning of each test is given in degree-days. Identical superscripts denote no significant difference in means of four to six observations.

Total Al added (μg/L)	pH	Survival to hatch (16 d)					Survival to 4-d posthatch (20 d)				
		0	50	100	200	300	0	50	100	200	300
Walleye age 160°d	6.0	99.0^{g} (1.5)	99.0^{g} (1.6)	—	—	—	90.3^{h} (8.3)	94.3^{h} (4.9)	—	—	—
	5.4	99.2^{g} (1.6)	98.5^{g} (3.7)	98.2^{g} (3.2)	—	—	96.3^{h} (2.9)	95.6^{h} (3.1)	68.6^{ef} (12.4)	—	—
	5.1	97.4^{g} (1.3)	98.1^{g} (2.3)	99.0^{g} (1.5)	84.9^{f} (7.0)	—	87.5^{gh} (9.0)	90.6^{h} (7.4)	62.8^{e} (13.3)	16.8^{c} (11.9)	—
	4.9	93.5^{g} (3.8)	92.9^{g} (3.4)	93.5^{g} (7.2)	62.4^{e} (3.0)	60.8^{e} (11.2)	78.1^{fg} (6.6)	34.3^{d} (15.4)	1.9^{ab} (3.5)	5.1^{b} (4.7)	0^{a} (0)
	4.5	12.2^{cd} (6.3)	15.5^{d} (7.2)	4.7^{bc} (3.9)	4.8^{bc} (3.5)	6.5^{bc} (6.9)	0^{a} (0)	1.0^{ab} (1.5)	1.0^{ab} (2.3)	0^{a} (0)	0.5^{a} (1.1)
	4.2	3.9^{ab} (5.9)	3.4^{ab} (2.2)	8.6^{bc} (9.2)	3.3^{ab} (4.4)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0.5^{a} (1.2)	0^{a} (0)	0^{a} (0)
				(5 d)					(9 d)		
Common Shiner age 54°d	6.0	98.5^{e} (2.9)	98.5^{e} (1.7)	—	—	—	94.1^{d} (8.3)	93.8^{d} (8.8)	—	—	—
	5.4	97.9^{e} (1.4)	94.9^{e} (6.5)	30.0^{d} (41.4)	—	—	97.1^{d} (0)	92.0^{d} (7.4)	0^{a} (0)	—	—
	5.1	94.9^{e} (4.4)	85.1^{e} (8.7)	10.9^{cd} (8.5)	0^{a} (0)	—	91.9^{d} (7.4)	82.3^{c} (6.3)	0.7^{a} (1.5)	0^{a} (0)	—
	4.8	11.7^{bcd} (10.5)	4.5^{abc} (2.6)	0^{a} (0)	2.3^{abc} (1.5)	1.4^{abc} (1.6)	8.0^{b} (6.6)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)
	4.5	0^{a} (0)	0^{a} (0)	0^{a} (0)	0.8^{ab} (1.5)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)
	4.2	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)
				(4 d)					(8 d)		
White sucker age 134°d	6.0	100^{g} (0)	98.6^{fg} (2.4)	—	—	—	99.0^{f} (1.5)	95.7^{f} (6.7)	—	—	—
	5.4	99.0^{g} (1.5)	100^{g} (0)	84.1^{ef} (10.0)	—	—	98.6^{f} (1.2)	95.2^{ef} (3.4)	71.5^{de} (17.2)	—	—
	5.1	99.5^{g} (1.2)	100^{g} (0)	82.7^{e} (8.7)	31.9^{cd} (9.6)	—	97.9^{f} (1.7)	99.0^{f} (1.5)	66.7^{d} (13.1)	8.3^{b} (2.9)	—
	4.8	91.4^{efg} (7.0)	45.7^{d} (9.3)	72.4^{e} (26.9)	30.4^{cd} (24.8)	9.0^{ab} (13.6)	79.7^{e} (5.2)	32.4^{c} (11.2)	11.3^{b} (13.0)	0^{a} (0)	0^{a} (0)
	4.5	14.4^{abc} (5.3)	10.7^{ab} (7.0)	16.0^{abc} (13.4)	24.2^{bcd} (17.9)	16.8^{abc} (12.2)	3.9^{a} (5.8)	1.4^{a} (3.5)	1.0^{a} (1.6)	0^{a} (0)	0^{a} (0)
	4.2	15.7^{ab} (16.7)	3.8^{a} (2.4)	12.5^{abc} (5.7)	10.3^{ab} (16.0)	4.7^{a} (5.3)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)
		Survival to 4-d posthatch (7 d total)					Survival to 8-d posthatch (11 d total)				
Smallmouth Bass age 54°d	6.0	97.3^{e} (2.1)	97.3^{e} (2.1)	—	—	—	97.3^{g} (2.1)	94.6^{fg} (6.1)	—	—	—
	5.4	97.3^{e} (3.3)	95.3^{e} (5.9)	98.0^{e} (3.3)	—	—	97.3^{g} (3.3)	94.7^{g} (5.5)	98.0^{g} (3.3)	—	—
	5.1	98.7^{e} (2.1)	98.7^{e} (2.1)	99.3^{e} (1.7)	98.0^{e} (2.2)	—	98.0^{g} (2.2)	98.7^{g} (2.1)	99.3^{g} (1.7)	98.0^{g} (2.2)	—
	4.8	98.0^{e} (2.2)	98.0^{e} (2.2)	96.7^{e} (4.7)	98.7^{e} (2.1)	98.0^{e} (3.3)	97.3^{g} (2.1)	98.0^{g} (2.2)	96.7^{fg} (4.7)	50.0^{d} (7.5)	24.7^{c} (13.5)
	4.5	61.8^{d} (12.9)	81.3^{d} (15.3)	98.0^{e} (3.3)	96.6^{e} (3.0)	93.2^{e} (2.3)	74.7^{ef} (20.7)	61.3^{de} (26.6)	53.1^{d} (21.2)		2.0^{ab} (2.2)
	4.2	45.3^{a} (10.7)	58.4^{ab} (12.7)	67.4^{bc} (14.8)	76.2^{cd} (5.7)	77.9^{cd} (10.3)	0^{a} (0)	3.4^{ab} (4.7)	6.8^{b} (5.7)		4.8^{b} (4.1)

TABLE 7. Effect of pH and Al on survival of fingerling Walleye (A), and fry stages of White Sucker (B), Lake Whitefish (C), and Smallmouth Bass (D) in soft water. Values given are mean (SD) of four to six observations and identical superscripts denote no significant difference in treatment means. Length of exposure is given in days and age at the start of each test is given in degree-days where data were available.

A. Walleye	pH	Total Al added (μg/L) 0	50	100	200	300
Fingerling 4 d exposure	6.0	89.9^{c} (9.6)	94.4^{c} (9.6)	—	—	—
	5.6	—	—	—	—	—
	5.0	80.9^{bc} (16.5)	100^{c} (0)	100^{c} (0)	18.4^{a} (8.8)	—
	4.7	80.9^{bc} (16.5)	80.9^{bc} (8.2)	80.9^{bc} (8.2)	66.7^{b} (21.8)	—
	4.5	57.1^{b} (0)	85.7^{c} (24.7)	95.2^{c} (8.2)	80.9^{bc} (8.2)	—
	4.2	—	—	—	—	—

B. White Sucker	pH	Total Al added (μg/L) 0	50	100	200	300
Yolk-sac fry age = 174°d 4-d exposure	6.0	100^{e} (0)	100^{e} (0)	—	—	—
	5.4	100^{e} (0)	100^{e} (0)	97.9^{de} (4.3)	—	—
	5.2	99.3^{e} (1.4)	100^{e}** (0)	96.4^{de}** (4.3)	68.3^{c}** (2.4)	—
	4.8	93.6^{d}* (2.7)	90.0^{d}* (3.7)	72.6^{c}* (9.0)	92.9^{d}* (4.9)	0^{a}
	4.5	5.7^{b}* (4.7)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)
	4.2	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)

C. Lake Whitefish	pH	Total Al added (μg/L) 0	50	100	200	300
Swim-up fry age = 800°d 12-d exposure	6.0	95.1^{e} (3.8)	—	—	—	—
	5.1	96.3^{e} (4.0)	74.3^{d} (9.4)	30.0^{c} (23.4)	—	—
	4.8	28.9^{c} (10.2)	28.1^{c} (15.7)	1.1^{a} (2.7)	0^{a} (0)	0^{a} (0)
	4.6	11.7^{b} (12.8)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)
	4.4	4.4^{ab} (10.9)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)
	4.2	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)

D. Smallmouth Bass	pH	Total Al added (μg/L) 0	50	100	200	300
Swim-up fry age = 180°d 7-d exposure	6.0	100^{e} (0)	98.0^{e} (2.2)	—	—	—
	5.4	99.3^{e} (1.7)	96.6^{e} (4.9)	94.7^{e} (4.8)	—	—
	5.1	95.9^{e} (2.6)	97.4^{e} (3.2)	94.0^{e} (7.5)	79.3^{d} (11.4)	—
	4.8	0^{a} (0)	0.6^{a} (1.6)	10.2^{b} (7.7)	26.9^{c} (11.8)	0^{a} (0)
	4.5	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)
	4.2	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)	0^{a} (0)

*All survivors moribund.
**15% of survivors moribund.

the LC50 for walleye cleavage eggs from pH 4.80 before hatching to pH 5.28 at 4 d posthatch, compared with pH 4.86 in the absence of Al (Table 4). For white sucker the LC50 increased from pH 4.47 at 4 d to pH 5.03 after hatching, compared with pH 4.72 in the absence of Al (Table 4).

Lethality of Al to eyed eggs, like that of low pH, became apparent during the hatching period and reduced survival of fry further in the 4-d period after hatch (Fig. 5B). Thus LC50s in the presence of 100 μg/L Al increased from pH <4.2 after 4 d of exposure of eyed eggs of walleye and white sucker to pH 5.05 and 5.02 at 4 d posthatch (Table 4).

Fry stages showed varying responses to Al. For fingerling walleye, 200 μg/L was lethal at pH 5.0 but 50 and 100 μg/L additions were beneficial at pH 4.5 (Table 7). Sac fry of white sucker were extremely sensitive to Al at pH ≥5.2 but low pH alone was lethal at pH ≤4.8 (Table 7). Aluminum was lethal to swim-up fry of smallmouth bass at pH 5.1 (200 μg/L) but was beneficial at pH 4.8 and 50–100 μgAl/L had no effect at pH 5.4 and 6.0 (Table 7). Survival of swim-up fry of largemouth bass exceeded 94% at pH ≥4.2 and Al ≤300 μg/L (data not shown).

The results of all tests are summarized in Table 8. The pH of extreme H^+ lethality in the absence of Al (survival ≤10%) ranged from pH <4.2 for eyed eggs of lake whitefish to pH 5.1 for cleavage eggs of common shiner. The onset of H^+ lethality (survival significantly reduced from that at pH 6.0) ranged from pH <4.2 to pH 5.4 for the same life stages of whitefish and common shiner. Al lethality was greatest, producing concentration dependent mortality, at pH 4.8–5.1 for all but the common shiner, but low pH was lethal in the absence of Al at pH 4.5–4.8 in the same tests. The pH of maximum Al effect was thus ≤0.3 pH units above that of the onset of lethality from low pH alone. For common shiner Al lethality occurred at pH 6.0 and low pH alone was lethal at pH 5.4, but pH levels between pH 5.4 and 6.0 were not tested.

Discussion

Our findings show that survival of early life stages of fish in acidified waters will be controlled primarily by their response to low pH. Al acted as an additional stressor but its toxicity was controlled by the sensitivity of individual species or life stages to low pH (Table 8). Maximum Al toxicity in all tests, except those on cleavage eggs of common shiner, occurred at pH 4.8–5.1. In all cases, the pH of maximum Al toxicity was either equal to, or 0.3 pH units greater than the pH which H^+ was lethal in the absence of Al. At pH ≤4.5 Al had either no effect or a temporary beneficial effect on survival.

Previous studies report maximum Al toxicity at pH 5.2–6.0 and suggest either joint action of low pH and IMAl to produce ionoregulatory failure, or asphyxiation by colloidal and polymeric forms at concentrations exceeding saturation (Driscoll et al. 1981; Neville 1985). Our results suggest that the first mechanism is more likely in natural waters. At pH 5.4 and 6.0, Al

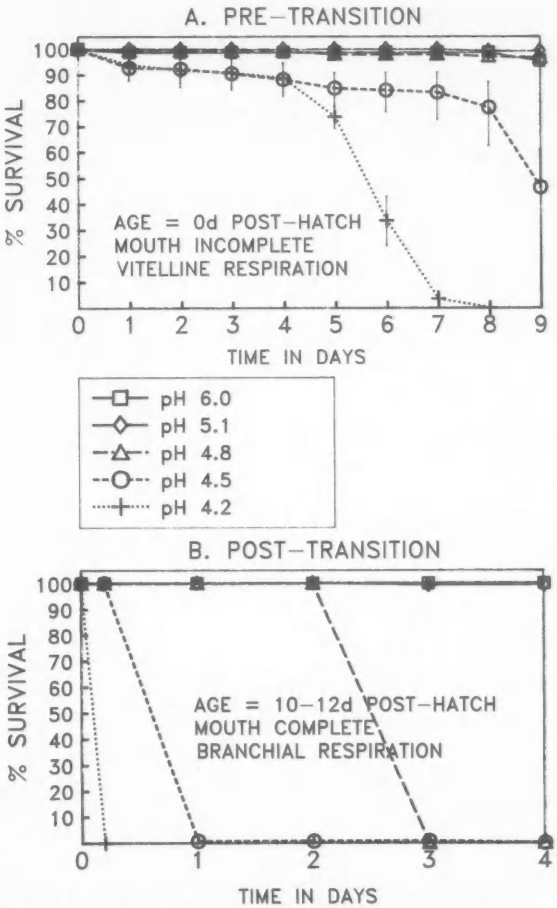

FIG. 3. Change in sensitivity of smallmouth bass fry to low pH coincident with transition from vitelline (A, pretransition) to branchial respiration (B, posttransition). Age at the start of each test is given for each stage and error bars enclose 1 SD about the mean of six observations.

was lethal only at concentrations of 100 and 50 μg/L, respectively (Tables 5 and 6). In common shiner tests, for example, pH 5.4 was lethal to cleavage eggs. Survival was increased when 50 μg/L Al were added but 100 μg/L decreased survival. At pH 6.0, survival was reduced when 50 μg/L Al was added. In both lethal treatments, total Al concentrations exceeded theoretical solubility (NRC 1986). Synthetic or treated test waters (i.e. Driscoll et al. 1981; Neville 1985) favour the existence of colloidal or polymeric Al forms when concentrations exceed solubility. In natural test waters such as ours, however, concentrations above solubility existed mostly as organic complexes (Fig. 1) with low toxicity (Driscoll et al. 1981; Hutchinson and Sprague 1987). Although we cannot conclusively discount the presence or toxicity of polymeric and colloidal Al in our tests, the fact that 17 μg/L of IMAl was not toxic to any species but the common shiner at pH 6.0, and that 24 μg/L of IMAl was beneficial to common shiner survival at pH 5.4 (Tables 3 and 5) both suggest that low pH was stressful and that IMAl added to this stress at pH 6.0, and so polymeric and colloidal Al forms were less important than low pH and IMAl

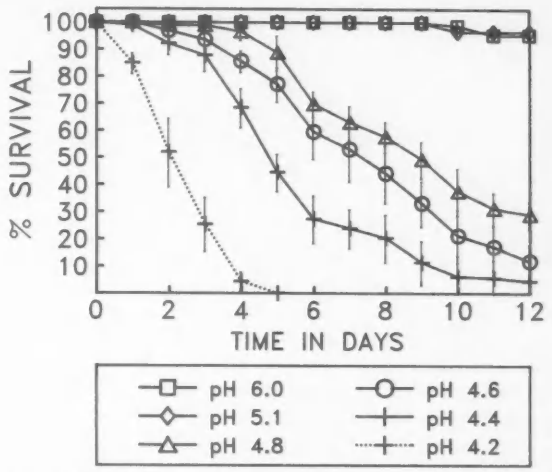

FIG. 4. Effect of length of exposure at different pH on survival of swim-up fry of lake whitefish. Error bars enclose 1 SD about the mean of six observations.

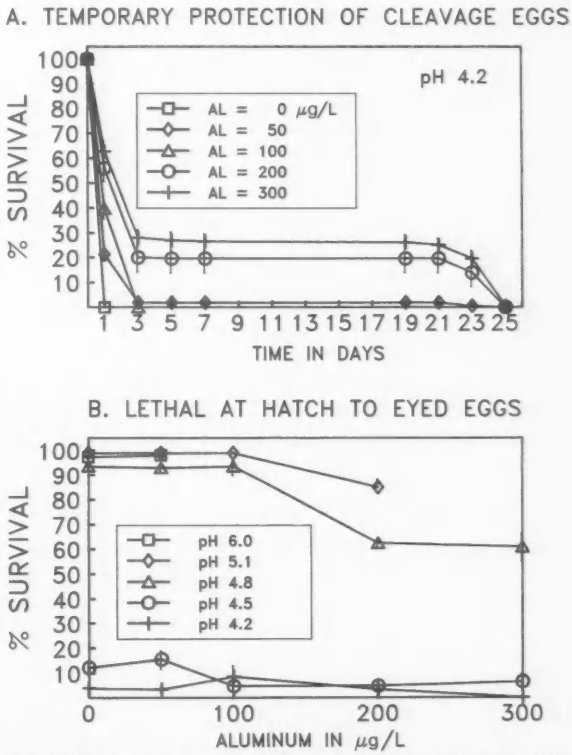

FIG. 5. Effect of Al on survival of two egg stages of walleye. (A) beneficial effect of Al on survival of cleavage eggs at lethal pH, (B) lethality of Al to eyed eggs at pH ≤4.8. Error bars enclose 1 SD about the mean of six observations.

to survival of the common shiner. In addition, the rapid replacement of our test water reduced the probability of Al polymerization (Hem and Roberson 1967).

It is therefore apparent that fish were responding to joint action of low pH and inorganic, monomeric Al at a pH between

TABLE 8. Effect of pH on lethality of inorganic, monomeric Al to early life stages of six native Ontario fish species. pH values are given for (A) highest pH where survival was ≤10% in 0 Al controls, (B) highest pH at which survival of 0 Al controls was significantly reduced ($p < 0.05$) from that at pH 6.0, and (C) pH at which Al additions produced significant reductions in survival over the concentration range tested. Species are identified as CS = Common Shiner, W = Walleye, WS = White Sucker, LWF = Lake Whitefish, SMB = Smallmouth Bass, LMB = Largemouth Bass.

Cleavage egg tests

Survival to	Eyed stage				Hatch				4-d posthatch			
	CS	WS	W	LWF	CS	WS	W	LWF	CS	WS	W	LWF
A	5.1	4.5	4.5	4.2	5.1	4.5	4.5	4.2	5.1	4.5	4.5	4.2
B	5.4	5.1	4.8	4.5	5.4	4.5	4.8	4.5	5.4	4.5	4.8	4.5
C	6.0	*	*	*	6.0	4.8	*	*	6.0	4.8	4.8/5.1	*

Eyed egg tests

Survival to	12 d	Hatch					4-d Posthatch				
	LWF	CS	WS	W	LWF	SMB	CS	WS	W	LWF	SMB**
A	<4.2	4.5	<4.2	4.2	<4.2	<4.2	4.8	4.5	4.5	<4.2	4.2
B	<4.2	4.8	4.5	4.5	<4.2	4.2	4.8	4.8	4.8	4.2	4.5
C	*	5.1	5.1	*	*	*	5.1	5.1	5.1	*	4.8

Fry tests

Survival to	4-d				
	CS	WS	W	LWF	SMB**
A	—	<4.5	4.5	4.4	4.8
B	—	4.8	4.8	4.8	4.8
C	—	5.1	*	5.1	*

* Al effect either beneficial or absent.
**8-d posthatch.

the lowest observed effect concentration and the no observed effect concentration for low pH alone (Table 8). This is in accord with previous laboratory testing of lake trout and brook trout (Hutchinson et al. 1987), rainbow trout (Neville 1985), amphibians (Clark and LaZerte 1985), and crayfish (Malley and Chang 1985). Field surveys from Scandinavia (Wright and Snekvik 1978) and the Adirondack mountains of the United States (Johnson et al. 1987) also showed that fish distributions were controlled primarily by surface water pH and that Al had a minor additional influence.

Our conclusions are not an artifact of increasing IMAl at low pH, nor of loss of Al from the exposure system. Average measured concentrations of total Al were never <89% of nominal levels. The IMAl fraction was more closely dependent on the total Al added than on pH within the lethal range of pH 4.8–6.0 and complexation of excess Al kept inorganic forms at concentrations close to or below solubility (Table 3, Fig. 1). Although we could have maximized Al toxicity by adding greater amounts at pH ≥5.1, LaZerte (1984) was not able to observe Al supersaturation in situ, could not induce Al precipitation by mxing acidic, Al-enriched water with lake water and concluded that laboratory toxicity tests using Al concentrations above saturation were not relevant to conditions in culturally-acidified lakes of south-central Ontario.

Cleavage egg sensitivity was established in the first 4 d of exposure and did not change through hatching. Eyed eggs, however, survived over most of the exposure but became sensitive at hatch (Fig. 2). Eyed eggs of lake trout also showed enhanced sensitivity to low pH in the 7 d preceding hatch (Hutchinson et al. 1989), and this phenomenon appears to be wide-spread (Peterson et al. 1982). The documented sensitivity of eggs and fry to both low pH and low oxygen tensions (Lacroix 1985) suggests that the increased sensitivity of eggs before hatching may reflect greater metabolic demands of active embryos. Delay in hatch and extension of the hatching period are thus responses to both hypoxia (Garside 1959), and to low pH (Fig. 2B).

For all species tested, eyed eggs exhibited greater tolerance to low pH than fry or cleavage eggs. Four-day LC50s represented 2.5 to 10-fold (0.3 to 1 pH unit) greater tolerance by eyed eggs (Table 4). Previous studies have also reported greater tolerance of low pH by eyed eggs in comparison to other early life stages for Pacific salmon (*Oncorhynchus* spp., Rombough 1982), Atlantic salmon *Salmo salar* (Daye and Garside 1980), smallmouth bass (Gannon and Werner 1982), white sucker (Trojnar 1977), and brook trout (Menendez 1976; Baker and Schofield 1982).

Greater tolerance of low pH by eyed eggs relative to fry could not be attributed to the zona radiata or perivitelline fluid (Daye and Garside 1977) as cleavage eggs were as sensitive as fry and tolerance of newly-hatched smallmouth bass was equivalent to that of eyed eggs. The egg capsule and perivitelline fluid may act as a barrier to Al transport, however, as shown by large increases in fry sensitivity to Al at hatch and absent or beneficial effects of Al to eggs for all species.

In sac-fry of smallmouth bass, maximum sensitivity coincides with the transition from vitelline to branchial respiration during the latter stage of yolk absorption (Daye and Garside 1980; Rombough 1982) and shows that fry sensitivity is equivalent to that of eggs for those species in which the transition to gill breathing is delayed. Similar developmental changes are reported for walleye (4 d posthatch at 15°C; McElman and Balon 1979), white sucker (4–6 d posthatch at 15°C; McElman and Balon 1980) and the common shiner (Leslie et al. 1986).

The absence of posthatch changes in common shiner sensitivity suggests that the branchial transition was delayed, as in

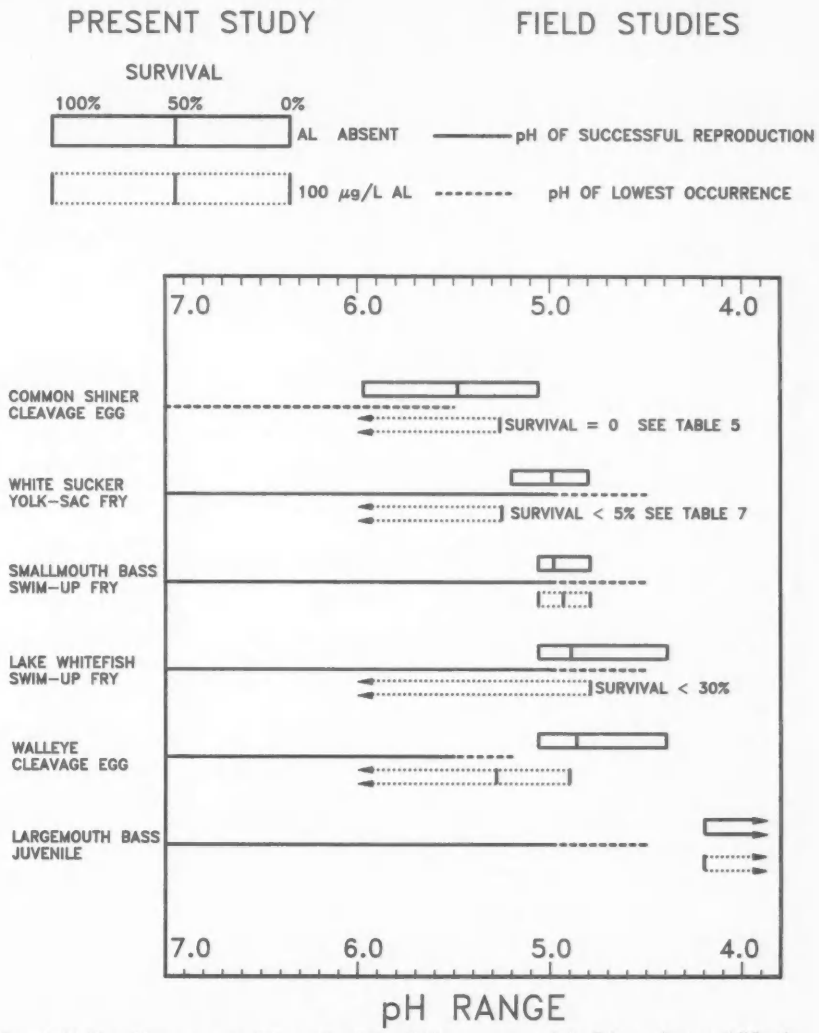

FIG. 6. Utility of laboratory toxicity testing in predicting response of six fish species to acidification. Box plots summarize response of most sensitive life stage of each species to pH and to pH + Al, as determined in the present study. Horizontal lines summarize pH of successful reproduction and lowest occurrence established from field surveys in eastern North America (Magnuson et al. 1984).

smallmouth bass, and that tests ending at 4 d posthatch were too short to observe the associated increase in sensitivity. Slight, but significant, increases in walleye and white sucker sensitivity to low pH within 4 d of hatch suggest that the branchial transition is gradual and that sensitivity would have been increased further in longer tests. The large increase in Al sensitivity of walleye and white sucker at hatch shows the loss of protection from the egg capsule and suggests partial gill development at this time, but does not exclude the possibility of higher mortality in longer tests.

It is possible that our test procedures underestimated the sensitivity of eggs to low pH. The LC50 for cleavage eggs of white sucker, for instance, was pH 4.72 and hatch success was not affected at pH $\geq$4.8. Trojnar (1977) reported no effect of pH $\geq$5.02 on survival of white sucker eggs from fertilization to hatch. Baker and Schofield (1982), on the other hand, observed significant mortality for cleavage eggs of the same species at pH $\leq$5.6 and variable egg mortality over the pH range 5.1 to 5.6. In the latter study, eggs were fertilized and water hardened in their respective treatments compared with water hardening at pH 6.8–7.1 for 2.5 h (Trojnar 1977) or 4 h (present study). Delayed transfer of desert pupfish (*Cyprinoden* n. *nevadensis*) eggs to acidified waters resulted in higher survival than transfer within 1 h of fertilization (Lee and Gerking 1980). Fertilization of eggs in treatment water may have increased the effects of acid exposure in the present study and more closely approximated conditions in situ.

Assessment of differences in physiological sensitivity between species and life stages requires comparison of equivalent responses over identical exposure periods. Our shortest test lasted 4 d and so the 4-d LC50 was used to compare phys-

iological tolerance (Table 4). A comparison of more ecological significance went beyond the 4-d period to assess survival to different developmental stages. This cumulative effect of chronic exposure was expressed as an LC50 and compared with the pH threshold for each species established from survey data to determine the validity of our laboratory estimates of tolerance (Fig. 6). The pH level of no observed effect provides an estimate of the pH of no chronic lethality and full environmental assessment requires knowledge of the concentration causing total mortality. Accordingly, the box plots in Fig. 6 enclose the range of pH over which survival decreased from no effect (no not significantly different from survival of pH 6.0 controls) to 50% (the LC50), to total effect (survival <10%), for the most sensitive life stage of each species tested. These laboratory data for pH, and for pH + Al were superimposed on the pH range over which each species is found in acid sensitive waters of eastern North America (Magnuson et al. 1984).

Our laboratory tests of early life-stage sensitivity to low pH alone provided adequate estimates of in situ distribution with pH for four of the six species tested (Fig. 6). White sucker, smallmouth bass, and lake whitefish showed recruitment failure in field surveys at a pH between laboratory estimates of no effect and the LC50. For the common shiner, the same effect levels bracketed the pH of extinction in situ. Sensitivity of the first three species was determined by the response of sac-fry and swim-up fry. For common shiner, cleavage eggs were the most sensitive life stage tested as our fry tests may have ended before maximum posthatch mortality.

With the exception of lake whitefish, these species spawn in late spring or early summer (Scott and Crossman 1973). Lake whitefish spawn in the autumn and fry emerge and swim up around the period of ice out (Cucin and Faber 1985). Since most snowmelt occurs before ice-out (Gunn and Keller 1986), any episodes of acidic runoff will occur during the more tolerant eyed-egg stage of lake whitefish. Average conditions are thus representative of the chemistry encountered by the most sensitive early life stages of common shiner, lake whitefish, white sucker and smallmouth bass and so chronic exposure to constant conditions in laboratory tests provided adequate estimates of their sensitivity in situ. This correspondence between effect levels also suggests that spatial variability of pH does not modify the response of those species by providing refugia.

Walleye and largemouth bass were more tolerant of reduced pH in our laboratory tests than in field surveys (Fig. 6). This discrepancy likely reflects our failure to consider the most sensitive life stage. For walleye, tests were ended prior to the transition to gill breathing. Sensitivity of walleye egg stages was intermediate between that of common shiner and white sucker, and therefore extended fry tests might have placed overall walleye sensitivity between that of common shiner and white sucker and improved the correspondence between laboratory and in situ estimates of tolerance. Largemouth bass were tested well after swim-up and their tolerance reflects that of juvenile fish.

Al increased the lethal pH for four of the six species tested (Fig. 6). For three of these species, however, the correspondence between laboratory results and in situ sensitivity was adequate without considering Al effects, suggesting that fish were either more sensitive in laboratory tests or that in situ estimates of sensitivity were inaccurate. Walleye sensitivity to Al in laboratory tests increased the pH of effect to the point where recruitment failure would be predicted in situ. The absence of treatments between pH 5.4 and 6.0, toxicity of Al at concentrations near saturation (100 μg/L) and sensitivity to pH + Al prevented establishing no-effect combinations for common shiner, white sucker, walleye, and lake whitefish (Fig. 6). Basing sensitivity on 50 μg/L additions produced no-effect levels of pH 5.2, 5.1, and 5.1, respectively for white sucker, lake whitefish, and walleye and no change for the common shiner (Tables 5 and 7).

Based on our lowest pH of no observed effect (Fig. 6), continued presence of the common shiner cannot be ensured in lakes acidified to pH <5.92. Our estimate corresponds closely to the pH 6.0 threshold for the species in Adirondack waters reported by Schofield and Driscoll (1986), pH 6.2 for Wisconsin waters (Rahel and Magnuson 1983), and pH 6.0 for Pre-Cambrian Shield waters in Ontario (J. E. Matusek, Ontario Ministry of Natural Resources, unpubl. data). The sensitivity of cyprinid fish species to acid stress is well documented on the basis of field work (Rahel and Magnuson 1983; Mills et al. 1987; Johnson et al. 1987; Schofield and Driscoll 1986), but with the exception of one species (fathead minnow, *Pimephales promelas* Mount (1973)) no laboratory estimates of pH tolerance have been published. Documented sensitivity, a short life span and wide distribution make the common shiner an ideal biological monitor of acidification.

The continued health of populations of walleye, white sucker, and lake whitefish cannot be ensured at pH <5.1 based on no-effect levels of pH 5.1, 5.11, and 5.16 for early life stages of each species, respectively. Addition of Al increased the pH of no observed effect to 5.4 and 5.34 for walleye and white sucker. This suggests that pH ≥5.4 is necessary for continued recruitment of these species in the presence of inorganic Al. Long-term survival of smallmouth bass cannot be ensured at pH <5.1. In the absence of complete testing on all life stages of largemouth bass, we recommend the same pH, based on identical pH sensitivities of these two species in situ (Fig. 6).

Minimum values of pH 4.09–4.85 are typical of 999 low-conductivity lakes (<40 μS/cm) in regions of Ontario receiving >50 $gm \cdot m^{-2} \cdot y^{-1}$ of atmospheric S loading, and mean pH was <6.0 for 659 of these lakes (Neary and Dillon 1988). It is clear that the correspondence between our laboratory estimates of sensitivity to low pH and in situ distribution along a pH gradient are sufficient to suggest recruitment failure of common shiner, walleye, lake whitefish, and white sucker in some of these lakes, even in the absence of solid documentation of loss. Mortality would be greater in lakes with elevated concentrations of Al and other trace metals (Hutchinson and Sprague 1986), with lower levels of Ca and ionic strength than in our test water (Hutchinson et al. 1987) and where episodic acidification impacted early life stages of fish (Gunn and Keller 1984). In spite of the influence of these modifying factors it is clear that pH is the primary factor governing recruitment of fish in acid-sensitive water, both through its actions as a toxicant and through increased mobilization of Al and other metals at low pH. Any human intervention to increase pH in affected lakes will have a beneficial effect on fish survival.

Acknowledgements

This work was funded by the Ontario Ministry of the Environment as part of the Acidic Precipitation in Ontario Study (APIOS). We wish to thank Bruce LaZerte and the staff of the Dorest Chemistry Laboratory for Al speciation and water chemistry analyses, Greg Mierle for substantial help in manuscript organization, John Munro, Trevor Pawson, Erica Brown, and Martha Thompson for technical assistance and advice, and Jenny Reed and Sheryl Gleave for patience with many revisions of the manuscript.

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Pennate Diatom *Nitzschia pungens* as the Primary Source of Domoic Acid, a Toxin in Shellfish from Eastern Prince Edward Island, Canada[1]

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An outbreak of food poisoning in Canada during autumn 1987 was traced to cultured blue mussels (*Mytilus edulis*) from the Cardigan Bay region of eastern Prince Edward Island (P.E.I.). The toxin, identified as domoic acid, had not previously been found in any shellfish and this outbreak represents the first known occurrence of human poisoning by this neurotoxin. A plankton bloom at the time of the outbreak consisted almost entirely of the pennate diatom, *Nitzschia pungens* f. *multiseries*, and a positive correlation was found between the number of *N. pungens* cells and the concentration of domoic acid in the plankton. *Nitzschia pungens* f. *multiseries* isolated from Cardigan Bay produced domoic acid in culture at levels (1 to 20 pg·cell^{-1}) comparable with values estimated for *N. pungens* in the plankton samples. Isolates of several Cardigan Bay phytoplankton, including the closely related species *Nitzschia seriata*, failed to produce domoic acid. Other *Nitzschia* spp. and two *Amphora coffeaeformis* isolates also failed to produce domoic acid. We conclude that *N. pungens* was the major source of the domoic acid in toxic mussels in eastern P.E.I. The recurrence, in November 1988, of a monospecific bloom of *N. pungens* and the presence of domoic acid in plankton and mussels reinforced this conclusion.

Une flambée d'intoxications alimentaires au Canada au cours de l'automne 1987 a été attribuée à la consommation de moules bleues (*Mytilus edulis*) cultivées dans la région de la Baie de Cardigan dans l'est de l'Ile-du-Prince-Edouard. La toxine, identifiée comme étant l'acide domoïque, n'a pas été retrouvée auparavant chez aucun mollusque et, à notre connaissance, c'est la première fois que cette neurotoxine cause une intoxication alimentaire chez des humains. Au moment de la flambée, on a observé une efflorescence de phytoplancton constitué presque exclusivement de la diatomée pennée *Nitzschia pungens f. multiseries*, et on a trouvé une corrélation positive entre le nombre de cellules de *N. pungens* et la concentration d'acide domoïque dans le phytoplancton. La souche de *N. pungens f. multiseries* isolée de la Baie de Cardigan a produit en culture de l'acide domoïque à des concentrations comparables aux valeurs observées pour *N. pungens* retrouvé dans les échantillons de phytoplancton, soit de 1 à 20 pg·cellule^{-1}). Des isolats de plusieurs types de phytoplancton de la Baie de Cardigan, y compris ceux de la variété étroitement apparentée *Nitzschia seriata*, n'ont pas produit d'acide domoïque. D'autres espèces de *Nitzschia* et deux isolats de *Amphora coffeaeformis* n'ont pas produit d'acide domoïque non plus. Nous concluons que *N. pungens* a été la principale source d'acide domoïque dans les moules toxiques dans l'est de l'Ile-du-Prince-Edouard. La récurrence, en novembre 1988, d'une efflorescence monospécifique de *N. pungens* accompagnée de la présence d'acide domoïque dans le plancton et les moules renforcent cette conclusion.

Received January 27, 1989
Accepted March 30, 1989
(JA026)

Reçu le 27 janvier 1989
Accepté le 30 mars 1989

Shellfish toxicity and its association with exceptional blooms of plankton has a long and established history (Taylor and Seliger 1979; Parker and Tett 1987). This association is particularly well defined in the case of paralytic shellfish poisoning, which is a recurrent phenomenon in the Bay of Fundy (White 1987; Martin and White 1988) and the eastern seaboard of the United States (Anderson and Morel 1979).

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An outbreak of food poisoning in Canada during November and December 1987 was traced to cultured blue mussels (*Mytilus edulis* L.) from the Cardigan Bay region of eastern Prince Edward Island (P.E.I.). Three deaths and 105 confirmed cases of acute intoxication that followed consumption of mussels from this area were observed (T. M. Perl et al., unpubl. data). The unique aspects of the toxic response in test mice used for bioassays, and the symptomology of hospitalized patients established that the intoxication was not caused by known paralytic or diarrhetic shellfish poisons produced by dinoflagellates. Symptoms of intoxication in humans included abdominal cramps, vomiting within the first few hours, and neurologic responses involving disorientation and memory loss that could persist indefinitely (Debonnel et al. 1988; T. M. Perl et al., unpubl. data). The term amnesic shellfish poisoning (ASP) has been proposed as an appropriate name for this clinical syndrome (T. M. Perl et al., unpubl. data).

In mid-December 1987, the toxin in mussels was identified as domoic acid (Bird et al. 1988; Wright et al. 1989), and procedures for its routine analysis were established (Quilliam et al. 1988; 1989; Wright et al. 1989). A review of events relating to the domoic acid problem is given by Bird et al. (1988) and by Couturier (1988), and a preliminary account of the field work in P.E.I. is reported in Bates et al. (1988). Physical oceanographic and meterological data for eastern P.E.I. preceding the outbreak are found in Drinkwater and Petrie (1988). Domoic acid had not previously been found in any shellfish and this episode represents the first known occurrence of human poisoning by this neuroexcitatory amino acid.

Knowing the source of the toxin is critically important to the Atlantic shellfish industry. If the source was a marine organism, it must have been a very abundant and an extremely active producer of domoic acid to account for the large amount, in excess of 6 kg, of the toxin in the ≈63 000 kg of mussels growing in the affected area (Wright et al. 1989). The only previously reported sources of domoic acid were the rhodophycean macroalgae, *Chondria armata* Okamura (Takemoto and Daigo 1958, 1960) and *Alsidium corallinum* C. Agardh (Impellizzeri et al. 1975), both in the family Rhodomelaceae. A congener of *C. armata, C. baileyana* (Montagne) Harvey occurs in the southern Gulf of St. Lawrence (Edelstein et al. 1974), and was indeed shown to contain domoic acid (Bird et al. 1988), suggesting that macroalgae were a possible source in the present instance. However, mussels are more apt to accumulate toxins from phytoplankton, their dominant food source. In early December 1987 surveys (L. A. Hanic) revealed an extensive, dense phytoplankton bloom in eastern P.E.I. Extracts of the plankton collected at this time were toxic to mice, and gave symptoms similar to those of extracts from toxic mussels. This led to the hypothesis that the dominant phytoplankton species in this bloom was the source of the toxin. Here we relate the occurrence and distribution of toxic shellfish in inshore waters of P.E.I. to the spatial and temporal abundance of phytoplankton species and to their domoic acid content. We explore the relationship among these data sets and investigate the de novo production of domoic acid in culture by various phytoplankters.

Materials and Methods

Shellfish Collection

Shellfish were obtained through the courtesy and cooperation of eastern P.E.I. mussel growers and several private individuals residing in the Cardigan Bay area, and from the Department of Fisheries and Oceans (Charlottetown, P.E.I.; Halifax, N.S.; Dartmouth, N.S.). A batch of processed mussels (shucked, steamed, and individually quick-frozen), harvested in early November 1987, was obtained from North Ocean Enterprises Ltd. (Souris, P.E.I.).

Macroalgal Investigations

Mussels, and netting used to culture the mussels, were examined for the presence of perennating basal residua of *C. baileyana* (Novaczek et al. 1987). For this examination, we used intact "socks" of live cultured mussels from the Cardigan area, removed from an affected site in early December 1987, and held for several weeks at a clean locale. Shells of these mussels were examined for basal discs of perennial algae. Although some epizoic algae may have been acquired at the new site, the original perennial flora from the Cardigan system would have persisted unless undue desiccation or thermal stress (Novaczek et al. 1987) had occurred during transport. Suspect shells were checked under a stereomicroscope; those selected for culture to verify the identity of attached algae were separated from the animal, cleaned of silt, and incubated at 15°C in sterile seawater at an irradiance of 40–50 $\mu E \cdot m^{-2} \cdot s^{-1}$ (16:8 h light:dark), and with gentle agitation by compressed air. Sections of nylon netting, 1 and 2 m from the top of the "sock," were cultured in the same manner. The seawater was replaced after 48 h with enriched seawater medium SWM-3 (McLachlan 1977), and cultures were maintained for 2 wk, sufficient for bases of *C. baileyana* to initiate growth of uprights (Novaczek et al. 1987).

Frozen one- and two-year-old mussels collected from Boughton River and Tracadie Bay from June through November 1987, were also examined under a stereomicroscope for young stages and basal discs of macroalgae.

Plankton Sampling

Plankton samples were collected from inshore and estuarine waters of P.E.I. (Fig. 1), either by towing or by pumping into a net with a 10- and 28-μm mesh size. The sites, timing, and method of sampling depended on local weather and ice conditions. Generally, samples were collected within a depth range of 1 to 3 m.

Plankton Analyses

Abundance of organisms in the plankton, particularly the pennate diatom *Nitzschia pungens* Grunow f. *multiseries* Hasle, the dominant taxon in the plankton bloom, was determined by light microscopy of slurries of plankton mounted on standard microscope slides. Random microscopic fields at 250× magnification were counted until a stable percentage abundance of *N. pungens* was achieved. More fields (up to 50) were counted when the incidence of *N. pungens* was lower, and higher magnification (400×) was used for denser preparations. Only pigmented cells were registered as part of the plankton; empty diatom frustules were regarded as detritus. At times, 20-μL aliquots taken with a wide-bore (1.5 mm) pipet tip were counted in toto. These abundance values were used to estimate relative volumes of *N. pungens* and other major phytoplankton species.

The identity of diatom species was verified by scanning electron microscopy (SEM). Plankton samples and digestive gland tissue of mussels (containing ingested plankton) were first acid-cleaned by the method of Boyle and Pickett-Heaps (1984), but

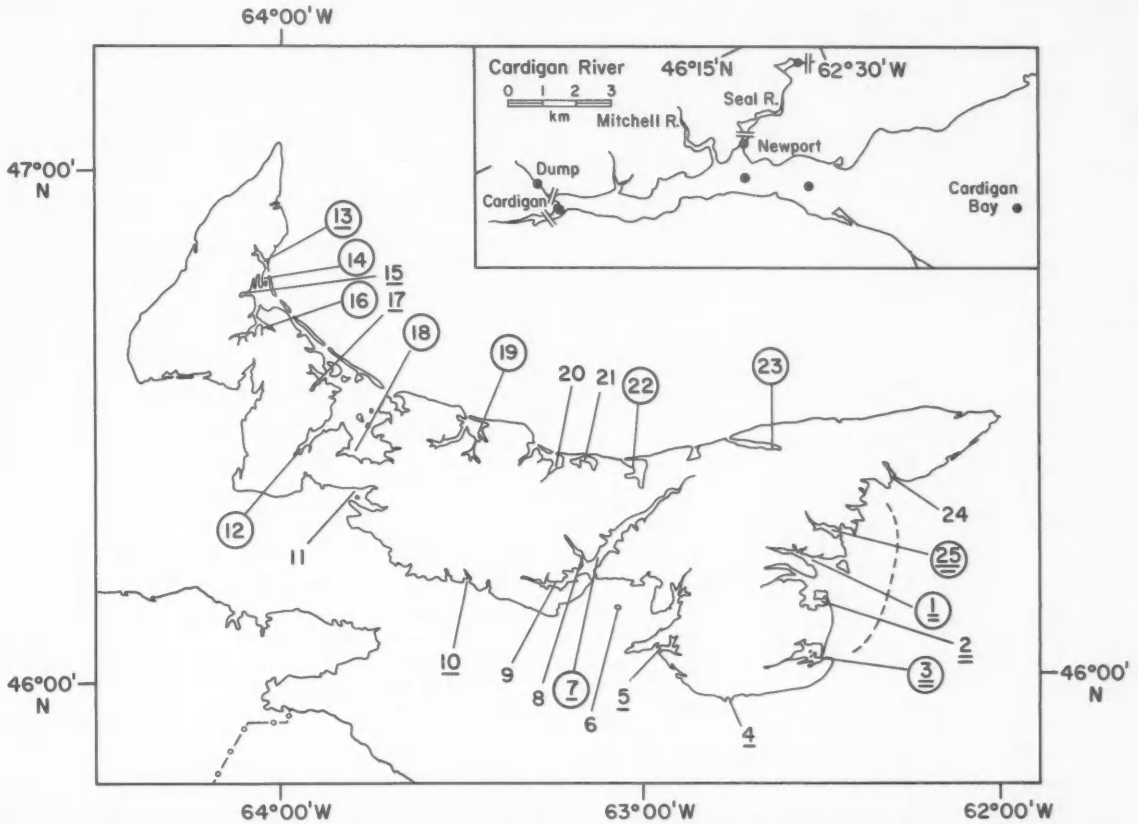

FIG. 1. Map of Prince Edward Island, showing sites where plankton was collected: (1) Cardigan River; (2) St. Marys Bay; (3) Murray River; (4) Wood Islands; (5) Pinette River; (6) Governor Island; (7) Hillsborough River; (8) North River; (9) West River; (10) Victoria Harbour; (11) Bedeque Bay; (12) Grand River (Malpeque Bay); (13) Kildare River; (14) Alberton; (15) Mill River; (16) Foxley River; (17) Bideford River; (18) Bentick Cove (Malpeque Bay); (19) New London Bay; (20) Rustico Bay; (21) Covehead Bay; (22) Tracadie Bay; (23) St. Peters Bay; (24) Souris River; (25) Boughton River. Most collections were made during mid-December 1987, to mid-January 1988; see Table 5 for specific collection dates of analyzed samples. Underlining signifies trace quantities or low percentages of *Nitzschia pungens* observed; double underlining, significant to large percentages. Circled numbers indicate samples analyzed for domoic acid. Broken line off the eastern edge of the island represents the seaward extent of *N. pungens* on 18 December 1987. Inset: detail of Cardigan River system, with sampling sites indicated by solid circles (see Table 5).

with boiling prolonged to 1.5 h to ensure complete removal of detrital organic matter. The clean diatom frustules were then concentrated on a 5-μm Nuclepore filter, washed with distilled water, sputter-coated with gold, and examined in a JEOL-35 SEM. Samples and SEM photomicrographs of *N. pungens* were sent to M. Poulin (National Museum of Natural Sciences, Ont.), G. A. Fryxell (Texas A&M University, TX), G. R. Hasle (University of Oslo, Norway), and D. G. Mann (University of Edinburgh, U.K.), who confirmed the identity.

The number of *N. pungens* cells present in plankton tow samples (cells per gram dry weight of plankton sample) that were analyzed for domoic acid was determined as follows. About 1 mg of dried sample was weighed to ±5 μg accuracy, and acid-cleaned as above. The clean residue, consisting of dissociated and fragmented diatom valves, was suspended in a known volume (usually 3 mL) of distilled water, and 10- or 20-μL aliquots were sampled for counting on the light microscope. Separate aliquots were taken for examination by SEM to assist in taxonomic identification and enumeration of the diatom valves. Four size classes (by length) of *N. pungens* fragments were used: <25, 25–50, 50–75, and 75–100%. Forty random fields were counted at 400× to ensure recognition of *N. pungens*. This covered about 0.8% of the area occupied by the sample on the slide and overcame the nonrandom distribution of particles in that additional counts did not significantly change the calculated cell density (*t*-test, $P>0.1$). Counts in each of these size-class fragments were converted to number of valves by multiplying the mean fractional length of the respective size classes (i.e. 0.125, 0.375, 0.625, 0.875) by the total number of fragments in each size class. Cell number was obtained by dividing the total for all classes by two. These cell number values permit calculation of the number of cells per unit dry weight of analyzed plankton, and the concentration of domoic acid per cell of *N. pungens* present in the plankton sample, assuming that all of the domoic acid in the sample was derived from *N. pungens*. The carbon content of the plankton was determined with a Perkin–Elmer Model 240C Elemental Analyzer on duplicate 1–3 mg subsamples of material that was used for domoic acid extraction.

TABLE 1. Microalgal species isolated from Cardigan Bay, eastern Prince Edward Island, and examined for the production of domoic acid.

Organism	Domoic acid
Nitzschia pungens f. *multiseries*[a]	+
Nitzschia pungens f. *multiseries*[b]	+
Nitzschia pungens f. *multiseries*[c]	+
Nitzschia seriata	ND[d]
Nitzschia frigida Grunow in Cleve and Grunow *prox.*	ND
Nitzschia microcephala Grunow in Cleve and Grunow	ND
Nitzschia pusilla Grunow emend. Lange-Bertalot	ND
Cylindrotheca closterium	ND
Amphiprora paludosa	ND
Amphiprora paludosa var. *hyalina*	ND
Chaetoceros septentrionalis	ND
Chlamydomonas sp.	ND
Ulothrix sp.	ND

[a]Isolate NPBIO, December 1987 (Subba Rao et al. 1988).
[b]Isolate NPARL, March 1988 (Bates et al. 1988).
[c]Isolate NPDFO, November 1988 (K. Freeman, Department of Fisheries and Oceans, Halifax, N.S.).
[d]ND = Domoic acid not detected (<1 ng·mL^{-1}).

Isolation and Culture of Phytoplankton

Major phytoplankton species in samples from the Cardigan area (Table 1), and especially from samples containing domoic acid, were isolated and grown in culture. *Nitzschia pungens* was isolated from plankton sampled in Cardigan Bay on 16 December 1987, on 14 March 1988, and on 1 November 1988. *Amphiprora paludosa* W. Sm. var., *hyalina* Cleve, and *Chlamydomonas* sp. were isolated from a domoic-acid-positive mixed culture initiated by J. Martin (Department of Fisheries and Oceans, St. Andrews, N.B.) from a Cardigan Bay sample of 16 December 1987. The remaining species were obtained from the Cardigan Bay sample of mid-March 1988.

Single cells or colonies, isolated by capillary pipette, were inoculated into f/2 medium (Guillard and Ryther 1962) made with filtered seawater obtained from the Aquaculture Research Station of the National Research Council of Canada, at Fink Cove, N.S. The concentration of Si in the growth medium was increased from 107 to 214 μM where indicated. Cultures of *N. pungens* f. *multiseries* and the very similar species, *N. seriata* Cleve, were each started from a chain of about 20 cells. The growth temperature was initially 5°C, and was gradually increased to 15°C. The irradiance level was 40, 100, 140, or 160 μE·m^{-2}·s^{-1}, depending on the experiment, and was provided by a bank of Cool-White fluorescent bulbs with a 14:10 h light:dark photoperiod. Irradiance was measured with a LI-COR Quantum Meter (Model LI-185B) fitted with a 4π sensor (Model SPH.QUANTUM).

Several related or otherwise suspect microalgae were tested for their ability to produce domoic acid. Twelve isolates of various *Nitzschia* spp., albeit not chain-forming ones, one of *Cylindrotheca* (formerly *Nitzschia) closterium* (Ehrenberg) Reiman and Lewin, and two isolates of *Amphora coffeaeformis* (C. Agardh) Kützing (Table 2) were acquired from the Provasoli–Guillard Center for Marine Phytoplankton (West Boothbay Harbor, ME). The latter species was chosen because a strain of *A. coffeaeformis* isolated by Y. Shimizu from toxic Cardigan River mussels was reported to produce domoic acid in culture (Shimizu et al. 1989). Cultures were grown in f/2 medium at 15°C and at an irradiance of ca. 90 μE·m^{-2}·s^{-1} with a photoperiod of 16:8 h light:dark, except for *Nitzschia* clones AA1, K3-3, and K8A, which were grown at 4°C and at the same irradiance and photoperiod. All cultures were harvested in the stationary phase for domoic acid assay.

Experiments to examine the growth characteristics and the ability of nonaxenic isolates of *N. pungens* and *N. seriata* to produce domoic acid were carried out under the culture conditions summarized in Table 3. Cell numbers were obtained by visual counts in two to four replicate 5-μL aliquots of culture.

Domoic Acid Analysis

Analyses of domoic acid concentration in aqueous extracts of shellfish tissue and plankton collected during autumn 1987 to spring 1988 were performed by reversed phase high performance liquid chromatography (HPLC) with ultraviolet diode array detection (1 μg·mL^{-1} detection limit) (Quilliam et al. 1988, 1989; Bates et al. 1988).

Cultures of *N. pungens* were analyzed for domoic acid with a high sensitivity (0.5 ng·mL^{-1} detection limit) 9-fluorenylmethoxycarbonyl chloride (FMOC) precolumn derivatization method for amino acids followed by reversed phase HPLC with fluorescence detection (Pocklington et al. 1989). To obtain the quantity of domoic acid produced by the whole culture, aliquots

TABLE 2. Diatom cultures obtained from the Provasoli–Guillard Culture collection, and examined for the production of domoic acid. Domoic acid was not detected (<1 ng·mL^{-1}) in any of these isolates.

Species	Clone	Origin
Nitzschia brevirostris Hustedt	01	Martha's Vineyard
Nitzschia curta (Van Heurck) Hasle	AA1	Antarctic
Nitzschia frustulum (Kützing) Grunow in Cleve and Grunow	13M	Nova Scotia
Nitzschia laevis Hustedt	72M	Woods Hole, MA
Nitzschia laevis	07	Martha's Vineyard
Nitzschia punctata (W. Smith) Grunow	52M	San Diego, CA
Nitzschia curvilineata Hustedt	5M	New Haven, CT
Nitzschia sp.	WD5	Gulf of Maine
Nitzschia sp.	CS106	Coral Sea, Australia
Nitzschia sp.	K3-3	Bering Sea
Nitzschia sp.	K8A	Kachemak Bay, Alaska
Nitzschia sp.	WINITZ	
Cylindrotheca closterium	NCLOST	Long Island Sound, NY
Amphora coffeaeformis	211M	Guam
Amphora coffeaeformis	47M	Woods Hole, MA

TABLE 3. Production of domoic acid (cells plus medium) in culture by three isolates of *Nitzschia pungens* from Cardigan Bay, eastern Prince Edward Island.

	Culture conditions			Conditions at stationary phase				
Isolate	Medium	Irradiance ($\mu E \cdot m^{-2} \cdot s^{-1}$)	Other	Plateau cell density ($cells \cdot mL^{-1}$)	Time to plateau (d)	Domoic Acid Production[a] ($pg \cdot cell^{-1}$)		
NPARL[b]	f/2[d]	165	10°C/10 L	36 400	18	0.2 (18)	0.7 (35)	0.9 (42)
NPARL	FE[e]	165	10°C/10 L	36 000	18	0.5 (18)	1.1 (35)	1.7 (42)
NPBIO[c]	f/2	165	10°C/10 L	37 100	18	0.1 (18)	0.3 (35)	0.3 (42)
NPBIO	FE	165	10°C/10 L	36 100	18	1.0 (18)	1.3 (35)	1.9 (42)
NPARL	f/2	100	10°C/1 L	82 900	14	0.04 (10)	0.7 (25)	—
NPARL	f/2	100	10°C/10 L	30 000	6	13.0 (17)	21.0 (21)	—
NPARL	f/2[f]	140	10°C/250 mL	129 300	11	1.3 (11)	10.4 (25)	18.0 (40)
NPARL	f/2[f]	40	10°C/250 mL	129 300	20	0.2 (11)	2.7 (25)	10.0 (40)
NPARL	f/2[f]	100	15°C/16 L	140 500	7	0.5 (7)	1.3 (17)	19.2 (28)
NPARL	f/2[f]	100	15°C/10 L	205 000	6	0.5 (6)	12.8 (12)	—
NPDFO[g]	f/2[f]	40	15°C/75 mL	170 000	6	1.4 (7)	11.5 (15)	13.7 (15)

[a]Values in parentheses refer to age of culture (in days) at time of sampling.
[b]NPARL = March 1988 isolate (Bates et al. 1988).
[c]NPBIO = December 1987 isolate (Subba Rao et al. 1988).
[d]Guillard and Ryther 1962.
[e]Subba Rao et al. 1988.
[f]Si concentration = 214 μM.
[g]NPDFO = November 1988 isolate (K. Freeman, Department of Fisheries and Oceans, Halifax, N.S.).

of the culture (cells plus medium) were sonicated for 1 min at 100 W using a 1-cm-diameter probe (Braun-sonic 1510) to disrupt the cells. Cell debris was then removed by filtration through a Millex-GS 0.22μm disposable filter (Millipore Corp.) prior to domoic acid analysis of this filtrate. Plankton samples collected during autumn 1988 to January 1989, were also analyzed by the FMOC method (Pocklington et al. 1989).

Confirmation of the identity of the domoic acid in mussel tissue, in plankton from P.E.I., and in *N. pungens* cultured in the laboratory was made by matching HPLC retention times, ultraviolet spectra, infrared spectra (Falk 1988), mass spectra (Thibault et al. 1989), and nuclear magnetic resonance spectra (Wright et al. 1989).

Results

Confirmation of the Identity of Domoic Acid

Proton nuclear magnetic resonance spectra (Fig. 2) of extracts from mussel tissue, plankton, and cultured *N. pungens* isolated from the affected area are essentially identical and are dominated by the characteristic resonances of domoic acid (Wright et al. 1989). Additional information on HPLC retention times, and infrared and mass spectra of domoic acid from plankton and mussel tissue are given in Bates et al. (1988).

Temporal and Spatial Distribution of Toxic Shellfish

Mussels containing measurable levels of domoic acid were confined to the Cardigan Bay region of eastern P.E.I. (Table 4). For example, domoic acid was observed in mussels only from Cardigan Bay and closely adjacent areas, such as Boughton River to the north and St. Marys Bay to the south. Domoic acid was undetectable in samples of various shellfish, including mussels, harvested during the summer and early autumn of 1987 (Table 4). The earliest appearance of domoic acid was from mussels harvested from the Cardigan River in late October. Analysis of fractionated tissue revealed that, in all cases, the toxin was localized in the digestive glands (Wright et al. 1989). The domoic acid concentration in extracts of the digestive gland reached a peak of 1500 μg domoic $acid \cdot g^{-1}$ wet weight in early November (Table 4). The domoic acid content then declined fairly rapidly in mussels harvested from mid-November 1987, to mid-January 1988, and the affected area was officially cleared for harvesting mussels, clams, and quahogs in April 1988. In November 1988, however, the domoic acid content of mussels from Cardigan Bay rose to levels in excess of 20 $\mu g \cdot g^{-1}$ wet weight, and mussel harvesting was again suspended.

Macroalgae Associated with Toxic Mussels

The algal flora on mussels from toxic areas did not differ noticeably from that on mussels from an unaffected area (Tracadie Bay). All shells were remarkably clean of algae, even those collected during the summer, the period of rapid growth for many macrophytic algae. Basal discs of *C. baileyana* were not seen on any of the shells examined, and cultures of fresh Cardigan shells failed to disclose the presence of perennating basal cells or minute young stages of this species. Culture netting was similarly negative for *C. baileyana*. In both fresh and frozen samples, identifiable macroalgae were small in stature, even those belonging to intrinsically larger species (e.g. *Giffordia sandriana* (Zanardini) Hamel, *Polysiphonia harveyi* Bailey).

Species Composition of the Plankton

From mid-December 1987 to mid-January 1988, the microplankton of Cardigan Bay, as sampled by a 20-μm mesh, consisted almost entirely of the pennate diatom, *N. pungens* f. *multiseries* (Tables 5 and 6; Fig. 3). This same species was brought into culture from samples collected in Cardigan Bay (Table 1). *Nitzschia pungens* occurred in chains of up to 38 cells (Fig. 4A), rarely singly, and was distinguished from 16 other chain-forming species of *Nitzschia*, including the similar and commonly reported *N. seriata*, by its bilateral symmetry, narrow (3–5 μm)

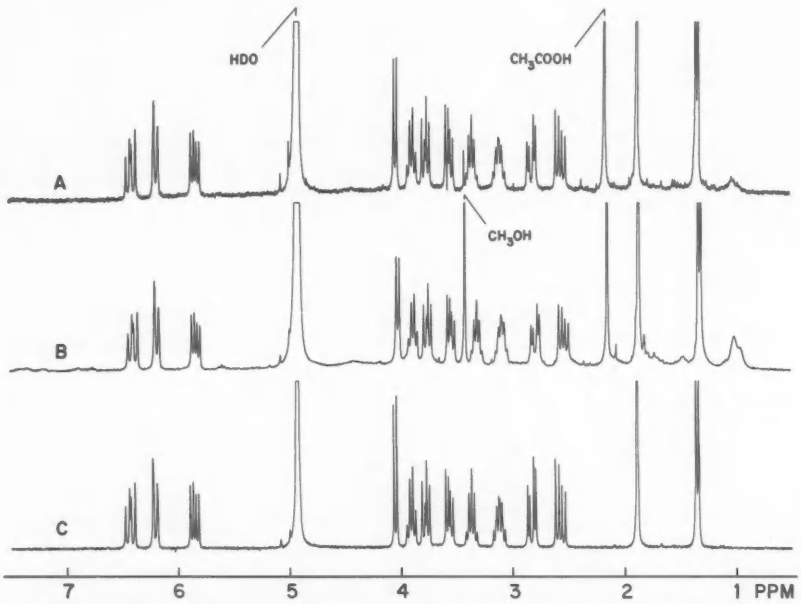

FIG. 2. ^1H nuclear magnetic resonance (solvent ^2H_2O, reference external TSP) spectra of domoic acid from: (A) Cardigan Bay plankton (20 December 1987); (B) *Nitzschia pungens* f. *multiseries* (NPARL) isolated from Cardigan Bay and grown in culture; and (C) Cardigan Bay cultured mussels (October 1987).

width, acute apices, lack of a central nodulus, and possession of 9–15 costae per 10 μm (Fig. 4B). By contrast, *N. seriata* is slightly asymmetric, broader than *N. pungens* (6 μm), and possesses 14–18 costae per 10 μm (Hasle 1965). As forma *multiseries*, the Cardigan diatom differed from the nominate form in having 3–4 rows of poroids between the costae, and spacing of poroids at 4–6 per 1 μm (Fig. 4C; Hasle 1965).

After mid-January 1988, the relative abundance of *N. pungens* declined in the central part of the Cardigan estuary (Seal River), and several species of the centric diatom *Rhizosolenia* Brightwell became increasingly common. By the end of February, *N. pungens* was practically absent, while *Rhizosolenia* remained dominant, accompanied by minor quantities of other centric diatoms and *Fragilaria* Lyngbye. It is of interest that *N. seriata* was found with *N. pungens* in the Cardigan River in late winter (March) but remained at a low density. A virtually monospecific but less dense population of *N. pungens* was also observed in Boughton River from mid-December to early January 1988. The Murray River plankton contained a smaller but still significant percentage of this diatom at about the same time (Table 5).

The number of cells of *N. pungens* per litre in the Cardigan system declined from ca. 15×10^6 on 11 December 1987, to 8×10^6 a week later, followed by a further decline to 2×10^6 in mid-January 1988, and then to $1–5 \times 10^4$ in mid-February. On 18 December 1987, the bloom extended ca. 10 km seaward from the mouth of the Cardigan River (Fig. 1). At this time *N. pungens* was found from Howe Point (46°18′N) to Cody Point (46°05′N), at which limits low (5450 cells·L^{-1}) to trace (55 cells·L^{-1}) concentrations of the diatom occurred. The decrease in bloom intensity and relative abundance of *N. pungens* coincided with a marked decrease in the concentration of total planktonic biomass. We emphasize, however, that the relative abundance of *N. pungens* reported here should not be construed as an accurate indicator of relative biomass, especially after mid-January when the plankton became increasingly dominated by diatoms much larger than *N. pungens*.

In autumn 1988, *N. pungens* f. *multiseries* reappeared in large numbers in the Cardigan River (Table 7), coinciding with a recurrence of domoic acid in mussels in this estuary and consequent suspension of harvesting in October. The diatom was observed in low numbers on 8 August, and its concentration had increased to ca. 4×10^5 cells·L^{-1} by 19 October, at which time it constituted nearly all the microplankton. This monospecific bloom condition continued until late December.

Domoic Acid in the Plankton

Plankton samples from autumn 1987 to spring 1988 (Tables 5 and 6) were taken with a 20-μm mesh, and do not represent the total population. Mouse bioassays of plankton extracts from early December 1987 produced symptoms similar to those obtained from extracts of toxic mussels, suggesting that the new toxin occurred in the plankton. Domoic acid was present, at a detection limit of 1 μg·g^{-1} wet weight, only in samples from a contiguous 30-km coastal region of eastern P.E.I. drained by the Cardigan, Murray and Boughton river systems (Fig. 1). The domoic acid concentration was highest (3000 to 12 000 μg domoic acid·g^{-1} dry weight) in samples collected from the Cardigan River in December 1987, and in early January 1988, when *N. pungens* constituted virtually all of the phytoplankton. Much lower levels (90 to 1560 μg domoic acid·g^{-1} dry weight) were found in samples from the Murray and Boughton rivers during December and January. The decrease in domoic acid concentration with time after mid-January was coincident with a decrease in both the absolute and relative abundance of *N. pungens*. This is illustrated by a positive correlation between the

TABLE 4. Domoic acid levels in shellfish[a] harvested from eastern Prince Edward Island.

Location	Date harvested	Shellfish type	Domoic acid ($\mu g \cdot g^{-1}$ wet wt)
Cardigan River	Fall-86	Quahogs[b]	ND[c]
Cardigan River	May-87	Mussels	ND
Cardigan River	Jun-87	Bar clams[d]	ND
Cardigan River	Jul-87	Bar clams[d]	ND
Cardigan River	Aug-87	Clams[e]	ND
Cardigan River	Aug-87	Clams	ND
Cardigan River	Sept-87	Clams	ND
Cardigan River	Oct-87 (last week)	Clams	ND
Cardigan River	Last week Oct. first week Nov. 1987	Mussels[f]	170[g]
Murray River	2-Nov-87	Mussels[h]	ND
Cardigan River	3-Nov-87	Mussels[h]	600
Cardigan River	6-Nov-87	Mussels[h]	790
		Whole tissue	770
		Digestive gland	1500
		Meat	420
Cardigan River	11-Nov-87	Mussels[h]	780
Cardigan River	16-Nov-87	Mussels[i]	
		Whole tissue	280
		Digestive gland	810
		Meat	50
Cardigan River	7-Dec-87	Clams	38
St. Marys Bay	7-Dec-87	Mussels	58
Boughton River	7-Dec-87	Mussels	22
Boughton Island	9-Dec-87	Mussels	190
Boughton River	10-Dec-87	Mussels	39
Cardigan River	10-Dec-87	Mussels[i]	
		Digestive gland	280
Cardigan River	14-Dec-87	Mussels	160
Hillsborough River	14-Dec-87	Mussels[j]	ND
Seal River	5-Jan-88	Clams	6
Cardigan River	5-Jan-88	Clams	6
Cardigan River	11-Jan-88	Mussels	30
Cardigan River	18-Jan-88	Mussels[j]	20
Tracadie Bay	18-Jan-88	Mussels	ND
Seal River	19-Jan-88	Clams	3
Cardigan River	19-Jan-88	Clams	1
Cardigan River	25-Jan-88	Mussels	20
Tracadie Bay	25-Jan-88	Mussels	ND
Cardigan River	1-Feb-88	Mussels	20
Cardigan River	1-Mar-88	Mussels	4
Cardigan River	2-Mar-88	Mussels	7
Cardigan River	3-Mar-88	Mussels	ND
Buctouche Harbour	4-Mar-88	Mussels	ND
Cardigan River	7-Mar-88	Mussels	ND
Buctouche Harbour	8-Mar-88	Mussels	ND
Buctouche Harbour	14-Mar-88	Mussels	ND
Tracadie Bay	24-Mar-88	Mussels	ND
Tracadie Bay	24-Mar-88	Mussels	ND
Cardigan Bay	24-Mar-88	Mussels	ND

[a]All shellfish samples were obtained frozen unless indicated otherwise.
[b]Canned.
[c]ND = Domoic acid not detected ($<1\ \mu g \cdot g^{-1}$).
[d]Bottled.
[e]Clam samples (*Mya arenaria*) were obtained shucked, steamed, and frozen.
[f]Pickled.
[g]Domoic acid values are for the whole mussel unless indicated otherwise.
[h]Obtained shucked, steamed, and individually quick-frozen.
[i]Mussels held in wet storage for 10 d depuration prior to analysis.
[j]Fresh.

concentration of domoic acid and the number of *N. pungens* cells in the net tow samples (Fig. 5).

It should be noted that the Murray River sample, collected on December 31, 1987, had a low concentration of domoic acid (90 μg domoic acid$\cdot g^{-1}$ dry weight), in spite of having a high relative abundance (70%) of *N. pungens*. However, the number of *N. pungens* cells was extremely low in this sample compared, for example, with the plankton collected only one day later from the Cardigan River (Table 6). It should also be noted that the Murray River sample consisted mostly of inorganic sediment particles with a relatively low carbon content of 8%, of which only about 3% was accounted for by *N. pungens* bio-

TABLE 5. percent abundance of *Nitzschia pungens* and other major constituents in the plankton of some inshore waters, P.E.I., mid-December 1987 to mid-march 1988; refer to Fig. 1 for locations of collection sites.

Location	Date	*N. pungens* abundance	Other common or dominant taxa
Cardigan Region			
Cardigan River[a,b]	11-Dec-87	>99	
Cardigan River[a,b]	15-Dec-87	>99	
Cardigan River[a,b]	16-Dec-87	>99	
Cardigan River[a,b]	20-Dec-87	100	
10 km offshore[a,b]	20-Dec-87	>99	
Cardigan River[a]	1-Jan-88	>99	
Cardigan Bay[a]	7-Jan-88	98	
Seal River[a]	18-Jan-88	77	*Rhizosolenia*
Seal River[a]	23-Jan-88	62	*Rhizosolenia*
Seal River[a]	24-Jan-88	43	*Rhizosolenia*
Seal River[a,c]	27-Jan-88	26	*Rhizosolenia*
Upper Seal River	13-Feb-88	0	*Licmophora*
Seal River[a]	14-Feb-88	12	*Rhizosolenia*
Dump site	14-Feb-88	0	*Fragilaria*
Seal River	26-Feb-88	<1	*Rhizosolenia*, other centric diatoms, *Fragilaria*
Seal River	9-Mar-88	<1	*Rhizosolenia*, other centric diatoms
Cardigan bridge	14-Mar-88	<1	*Rhizosolenia, Thalassiosira*
Murray River[a,c]	31-Dec-87	69	*Rhizosolenia, Thalassiosira, Thalassionema*
Boughton River[a]	2-Jan-88	93	
Hillsborough River	6-Jan-88	0	*Rhizosolenia, Thalassiosira*
St. Peters Bay	11-Jan-88	0	Oligotrichida (tintinnid loricae), Crustacea
Tracadie Bay	12-Jan-88	0	*Biddulphia*, Oligotrichida
New London Bay	13-Jan-88	0	Crustacea, Oligotrichida
Alberton	15-Jan-88	0	Crustacea
Malpeque Bay (Bentick Cove)	15-Jan-88	0	Crustacea, Oligotrichida
Foxley River	16-Jan-88	0	Crustacea, Oligotrichida
Malpeque Bay (Grand River)	17-Jan-88	0	Crustacea, Oligotrichida
Kildare River	18-Jan-88	<1	mostly detritus; no clearly dominant taxa

[a]Sample contained domoic acid (see Table 6).
[b]Mouse bioassay positive.
[c]Mouse bioassay negative.

TABLE 6. Domoic acid content of plankton tow samples from eastern Prince Edward Island as related to the presence of *Nitzschia pungens*.

		N. pungens in plankton		Domoic acid in plankton	Domoic acid in *N. pungens*	Total carbon in sample	*N. pungens* carbon in sample	Carbon accounted for by *N. pungens*
Location	Date	A abundance (%)	B (cells·g^{-1} dry wt) ($\times 10^{-6}$)	C (μg·g^{-1} dry wt)	D=C/B (pg·cell^{-1})	E (%)	F=B × f[a] (%)	G=F/E (%)
Cardigan River	16-Dec-87	>99	870	2890	3.3	15	6.1	41
Cardigan River[b]	20-Dec-87	100	2990	10620	3.6	22	21.0	95
Murray River	31-Dec-87	69	32	90	2.9	8	0.2	3
Cardigan River	1-Jan-88	>99	2130	11900	5.6	21	15.0	71
Boughton River	2-Jan-88	93	885	1560	1.8	22	6.2	28
Cardigan Bay	7-Jan-88	98	890	1110	1.2	15	6.2	42
Seal River	18-Jan-88	77	64	170	2.7	15	0.5	3
Seal River	24-Jan-88	43	177	460	2.6	22	1.2	6
Seal River	27-Jan-88	26	110	500	4.5	15	0.8	5
Seal R. bridge	14-Feb-88	12	60	40	0.6	18	0.4	2
Cardigan dump	14-Feb-88	0	0	1[c]	N/A[d]	18	N/A	N/A
Cardigan bridge	14-Mar-88	<1	25	5	0.2	22	0.2	1

[a]f has the value of 70 pg carbon·cell^{-1}, determined by converting cell volume measurements of *Nitzschia pungens* to carbon values (Strathman 1967). This estimate falls within the range of 52–187 pg carbon·cell^{-1} obtained by carbon analysis of *N. pungens* cultures of various ages.
[b]A second plankton tow sample collected ca. 10 km offshore from the Cardigan River, was >99% *N. pungens* and contained 1600 μg domoic acid·mL^{-1} of plankton concentrate.
[c]At the detection limit of the technique.
[d]N/A = not applicable.

mass (Table 6). Other diatom genera such as *Thalassiosira* Cleve, *Navicula* Bory, and *Rhizosolenia* were present (Bates et al. 1988), and could have accounted for most of the carbon in the sample. The carbon content of all the other samples was much higher and showed relatively little variation, ranging from 15 to 22% (column E, Table 6). But, as expected, the proportion of carbon accounted for by *N. pungens* decreased during December 1987 to March 1988, from a high value of 95% to low values of 3% or less (column G, Table 6), dependent on the number of *N. pungens* cells in the sample.

Estimates of domoic acid per cell, obtained by assigning the domoic acid content of the sample to the number of cells in the sample, resulted in remarkably similar values, ranging from 1 to 6 pg domoic acid·cell^{-1} for samples collected during the 6-wk period from mid-December 1987, to the end of January 1988 (column D, Table 6). Samples collected in February and March had much lower values. However, the error associated with these latter values is probably quite large owing to the very low incidence of *N. pungens*, and the correspondingly low levels of domoic acid in these samples. Analyses of the plankton bloom that occurred in autumn 1988 (Table 7), indicate that the domoic acid content of *N. pungens* in the Cardigan River ranged between 2 and 7 pg·cell^{-1}, similar to that reported for autumn 1987 (Table 6).

Survey of Phytoplankton for the Production of Domoic Acid

Domoic acid was undetectable (<1 ng·mL^{-1}) in stationary phase cultures of 10 phytoplankton species isolated from Cardigan Bay (Table 1). Likewise, domoic acid was undetectable in 12 *Nitzschia* species, one of *Cylindrotheca*, and two isolates of *Amphora coffeaeformis* from the Provasoli–Guillard Culture Collection (Table 2).

Examination of cultures of *N. pungens* and *N. seriata*, a pennate diatom similar in morphology to *N. pungens* and also found in eastern P.E.I. in late winter, provided additional information on growth rates and ability to produce domoic acid. The growth rate of *N. pungens* ranged between 0.64 and 0.71 divisions per day at 10°C and at 100 μE·m^{-2}·s^{-1} (14:10 h light:dark) (Fig. 6). The final cell yield during stationary phase was somewhat variable, ranging between 3×10^4 and 8×10^4 cells·mL^{-1} for growth in f/2 medium containing 107 μM Si, and 1.4–2.1×10^5 cells·mL^{-1} for growth with 214 μM Si (Table 3). Domoic acid production by *N. pungens* was detected only during the stationary phase, and increased with time from the start of this phase (Fig. 6; Table 3). Even in late stationary phase (= >40 d), production of domoic acid was quite variable, with values ranging between 0.3 and 18.0 pg·cell^{-1}. The growth rate of *N. seriata* was 0.62 divisions per day at 10°C and at 100 μE·m^{-2}·s^{-1} (Fig. 6), similar to that of *N. pungens*. In contrast to *N. pungens*, domoic acid was undetectable (<1 ng·mL^{-1}) in cultures of *N. seriata* (Fig. 6).

Discussion

The toxic shellfish incident documented here is unique in that a diatom is identified as the most likely source of a naturally occurring toxin, whereas most of the documented cases of shellfish toxicity have involved dinoflagellates (Taylor and Seliger 1979). The event is also unique in that it is the first known occurrence of human intoxication due to ingestion of domoic acid. The rapid identification of domoic acid as the toxic agent (Wright et al. 1989) was crucial in deciding that the toxin was not man-made or of terrestrial origin, but was a naturally-occurring compound most likely produced by one or more organisms in the marine food web.

The dense bloom of phytoplankton in the affected area suggested that phytoplankton may have acted as the contaminated food vector that caused the accumulation of toxic levels of domoic acid in the mussels. The positive mouse bioassays of plankton extracts strongly reinforced this hypothesis, although there was no evidence in the literature that domoic acid was produced by any phytoplankton species. However, two species of benthic macroalgae (*Alsidium corallinum* and *Chondria armata*) were known to contain domoic acid (Takemoto and Daigo 1958, 1960; Impellizzeri et al. 1975). The available information on their distribution rules out both species as potential sources of domoic acid in eastern P.E.I. However, a related species, *C. baileyana*, is found in Malpeque Bay on the northern coast of P.E.I. (Fig. 1) and in similar embayments in the southern Gulf of St. Lawrence.

It should be kept in mind that bacteria or fungi could also be involved in domoic acid production. These organisms play a central role in habitat ecology, not only as decomposers but also as important sources of dissolved organic compounds. Such compounds could act as growth stimulants or as synergists in the de novo production of domoic acid by macroalgae as well as by phytoplankton.

Possible Macroalgal Sources

Dried herbarium specimens (NRCC 6058) of *C. baileyana*, collected in 1973 from Malpeque Bay (Fig. 1), contained detectable amounts of domoic acid (Bird et al. 1988). This alga has not been recorded from eastern P.E.I., probably for lack of observation, as apparently suitable habitat occurs there. However, owing to its rarity in the Gulf of St. Lawrence and its relatively small size (filiform, <15 cm tall), *C. baileyana* is unlikely to achieve sufficient biomass in the affected estuaries to form a major component of the mussel diet. In the Gulf, this species is thermally limited to one, occasionally two, generations per year (Novaczek et al. 1987) and, despite its ability to perennate via basal residua, would require several years to develop an abnormally large biomass. If *C. baileyana* were the chief source of domoic acid in eastern P.E.I. waters, an early, low-level toxicity would probably have become apparent at least a year before the 1987 episode. Moreover, such a large well-established population would have been visible on the mussel culture systems, which provided a significant increase in the amount of solid substrata available for colonization by algae.

Even had large quantities of *C. baileyana* been present, it is unlikely that the mussels could have acquired all their domoic acid content by direct consumption of algal detritus and spores. Autumn dieback of *C. baileyana* occurs by progressive cellular senescence and decay in situ, and fragments containing intact cells would not be liberated at any given time in sufficient quantity for capture by mussels to account for the high concentrations observed. Although mussels process huge volumes of water relative to their body size (Drinnan 1964), it is unlikely that the P.E.I. shellfish acquired their burden of domoic acid by this vector since domoic acid released into the water by cellular disintegration of *C. baileyana* would result in only extremely low levels for direct uptake. Moreover, domoic acid has a high solubility in water and a very low solubility in hydrocarbon solvents (M. Falk and J. Walter, in prep.), which indi-

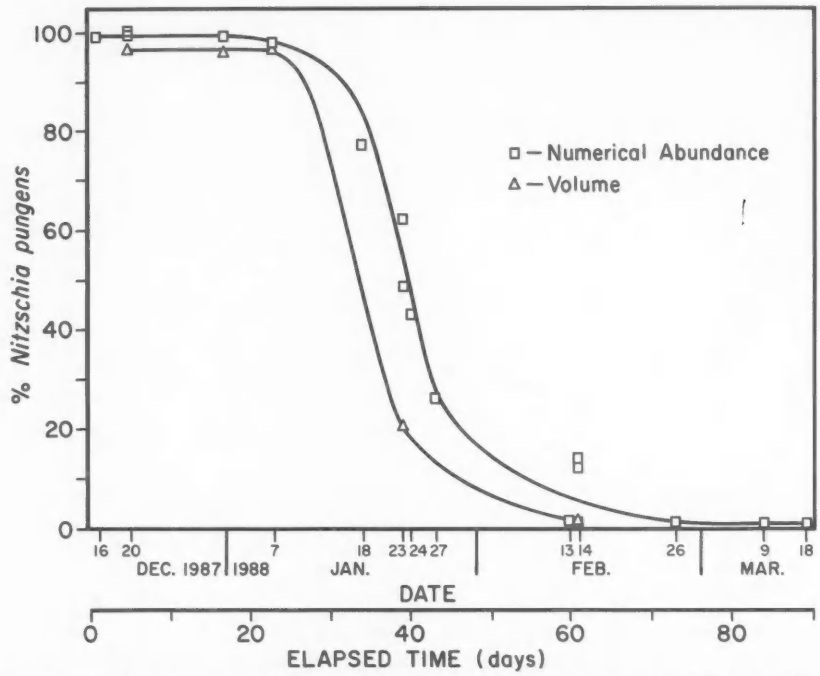

FIG. 3. *Nitzschia pungens* f. *multiseries* as a constituent of the phytoplankton in the Cardigan Bay system.

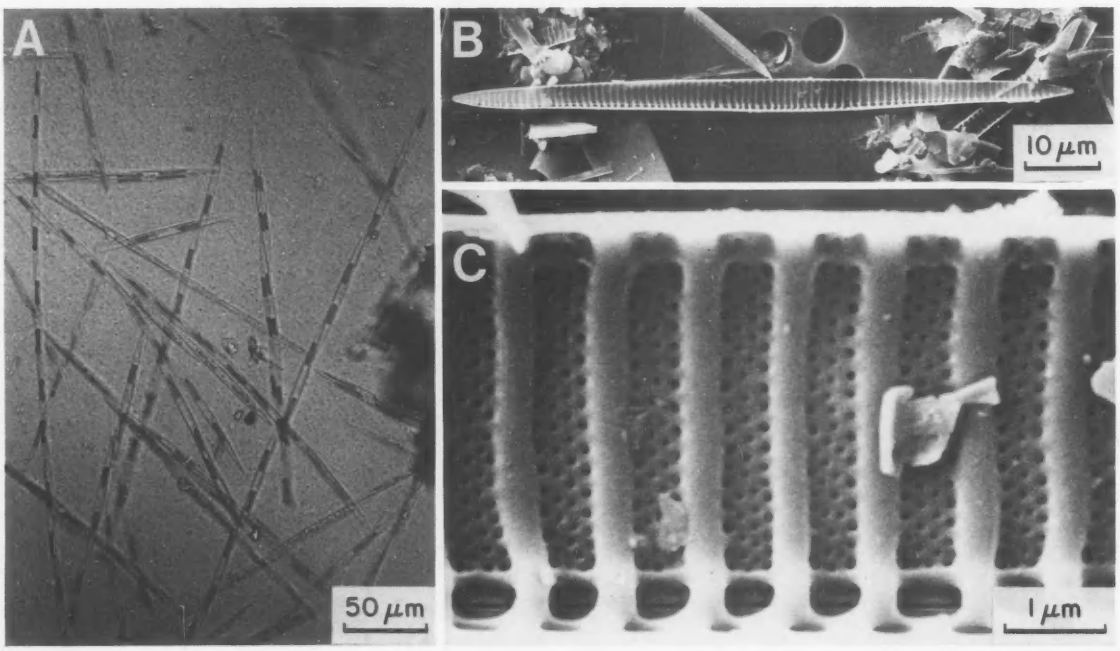

FIG. 4. *Nitzschia pungens* f. *multiseries* from Cardigan Bay: (A) light photomicrograph showing chains of *N. pungens*; (B) scanning electron micrograph of a single valve showing the costae; and (C) same valve at higher magnification, showing the rows of poroids between the costae.

TABLE 7. Abundance and domoic acid content of *Nitzschia pungens* from Cardigan Bay; autumn–winter, 1988–1989.

Date	Abundance[a] (%)	Concentration[b] (cells·L^{-1}) × 10^{-3}	Domoic acid[a] (pg·cell^{-1})
13-Oct-88	28	61	3.7
19-Oct-88	86	359	2.3
24-Oct-88	90	273	3.7
1-Nov-88	96	181	3.4
7-Nov-88	97	231	6.1
14-Nov-88	97	979	5.2
23-Nov-88	99	1260	6.1
30-Nov-88	98	658	7.1
12-Dec-88	98	598	2.2
20-Dec-88	98	413	2.8
28-Dec-88	89	84	2.8
3-Jan-89	17	<3	ND[c]
10-Jan-89	13	<0.4	ND

[a]In concentrates obtained with a 20-μm mesh net.
[b]In unprocessed seawater.
[c]ND = Domoic acid not detected in plankton concentrate (<1 ng·mL^{-1}).

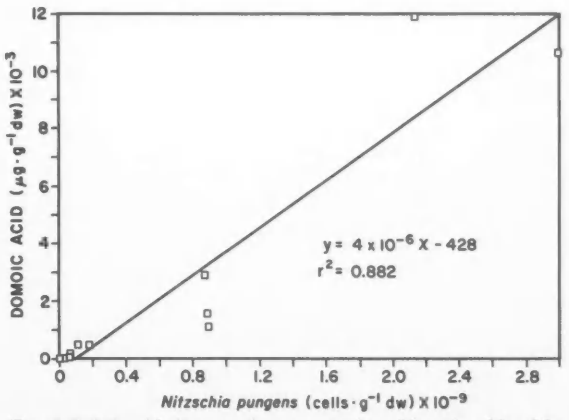

FIG. 5. Relationship between the concentration of domoic acid and the number of *Nitzschia pungens* cells in net plankton tow samples from the Cardigan Bay system.

cates that its association with, and transport across, lipophilic biological membranes would not be an efficient process.

It is quite probable that other macroalgae produce domoic acid, particularly species of the genus *Chondria*. However, we can conclude with certainty that macroalgae were not the major source of domoic acid in the Cardigan River region.

Possible Phytoplankton Sources

It is clear from our observations that appreciable amounts of domoic acid must have been present in the affected area as early as middle to late October, probably reaching maximum values during November and December 1987. The total amount of domoic acid in all cultivated mussels at this time, estimated at about 6 kg (Wright et al. 1989), must have represented only a very small fraction (probably 1% or less) of the total domoic acid production in the affected area. We can expect, therefore, that the total production of domoic acid could have been as much as 1000 kg or more. The only obvious potential source of this much domoic acid was the intense and persistent bloom

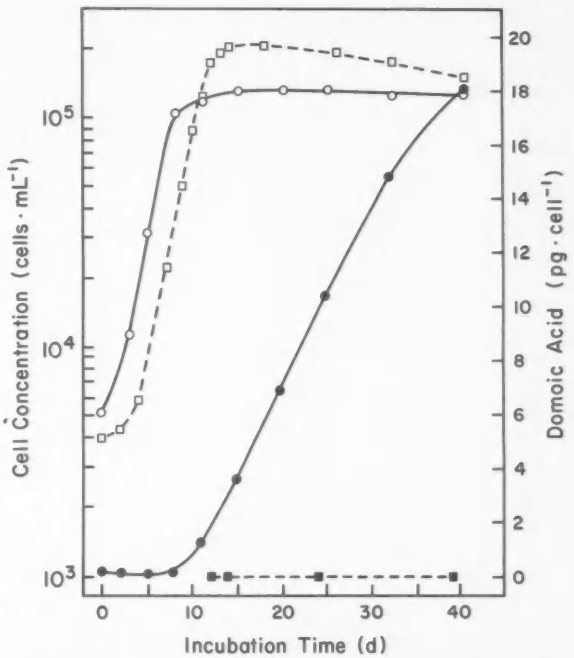

FIG. 6. Growth curve of *Nitzschia pungens* (○), and *Nitzschia seriata* (□) isolated from Cardigan Bay, March 1988, and production of domoic acid by *N. pungens* (●). Domoic acid was undetectable (<1 ng·mL^{-1}) in *N. seriata* (■). Temperature = 10°C; irradiance = 100 μE·m^{-2}·s^{-1}, 14:10 h light:dark; culture volume = 1 L of f/2 medium (Guillard and Ryther 1962).

of phytoplankton dominated by the pennate diatom *Nitzschia pungens* f. *multiseries*.

All toxic mussels harvested from the Cardigan region in December 1987, showed engorged digestive glands containing identifiable frustule fragments of *N. pungens*. This was also true of toxic mussels harvested in October and November 1987, but no information is available on the plankton composition in the Cardigan at that time. However, phytoplankton samples collected in the Boughton River estuary from early September to mid-November 1987 showed a relatively high abundance of *N. pungens* (Johnson 1988). We can speculate that this diatom occurred in similar abundance in Cardigan Bay in September, and that it could have formed a significant part of the mussels' diet from late summer through autumn 1987, as indeed was the case in 1988.

Our observations on the spatial and temporal relationships between the abundance of *N. pungens* and the toxic levels in mussels provides strong circumstantial evidence that this diatom was the major source of domoic acid. When injected into mice, extracts of plankton samples dominated by *N. pungens* (Table 5) produced symptoms identical to those produced by extracts of toxic mussels. Domoic acid in plankton was often in excess of 1% dry weight, sufficient to account for the toxicity of the plankton extracts to mice, and for the levels of domoic acid observed in toxic mussels. Our calculations for both 1987 and 1988 indicate that the domoic acid content of *N. pungens* from the Cardigan River was from 1 to 6 pg·cell^{-1}. A level of 3 pg per cell represents a very large intracellular pool (2 to 3% of the total cellular carbon) of a single metabolite, particularly

in the case of a diatom the size of *N. pungens*. Mussels feeding mainly on *N. pungens* containing this much domoic acid would readily accumulate sufficient toxin in their digestive glands to account for the levels reported in Table 4. The conclusion that *N. pungens* was the source of the domoic acid is strengthened even further by (a) the detailed analysis of the plankton samples (Table 6), which demonstrates clearly that the domoic acid content of plankton samples from the affected area must have been associated with the *N. pungens* component of the plankton, (b) the recurrence of domoic acid-containing *N. pungens* in Cardigan Bay in late autumn 1988, and (c) the demonstration of de novo production of domoic acid by cultures of *N. pungens* (isolate NPBIO) from the affected area (Subba Rao et al. 1988).

Our results (Table 3) confirm that domoic acid is produced by three isolates of *N. pungens* f. *multiseries* originating from Cardigan Bay plankton collected in mid-December 1987 (NPBIO), mid-March 1988 (NPARL), and early November 1988 (NPDFO), when the abundance of this organism was >99, <1, and 96%, respectively, of the microplankton. The quantity of domoic acid produced per cell of *N. pungens* in culture is in the same range as that estimated for *N. pungens* cells collected in net plankton tows from P.E.I. during both the 1987 and 1988 episodes. The ability of the three isolates to produce domoic acid in very different culture media and at a variety of irradiance levels and temperatures (Table 3) confirms the potential of this diatom to be a major source of domoic acid in the environment.

The conclusion that *N. pungens* was the major source of domoic acid in eastern P.E.I. is underscored by our observation that other phytoplankton species isolated from Cardigan Bay, including *N. seriata*, failed to produce domoic acid in culture (Table 1). Shimizu et al. (1989) reported that the pennate diatom *Amphora coffeaeformis* isolated from toxic mussels from the Cardigan River, also produces domoic acid. We did not observe domoic acid production in two strains of *A. coffeaeformis* from the Provasoli–Guillard Center for Culture of Marine Phytoplankton, and 12 species of *Nitzschia* and one of *Cylindrotheca* from the same collection proved similarly negative (Table 2). We also emphasize that domoic acid was never detected in plankton samples that lacked *N. pungens*, and that *A. coffeaeformis* was not observed in domoic-acid-positive samples. In addition, surface sediment sampled from the affected area contained many *N. pungens* but no *A. coffeaeformis* frustules.

Little is known about the autecology of *N. pungens*. It is distributed almost world-wide in coastal waters (Hasle 1965, 1972), but statements on its temperature and salinity preferences are based largely on distributional records. We are not aware of any previous reports of this diatom forming dense, persistent unialgal blooms. If *N. pungens* was present at bloom levels in eastern P.E.I. as early as October 1987, as seems to have been the case, it is difficult to explain why this bloom persisted and intensified over the 3-mo period from October to December. A possible factor is the insecticidal property of domoic acid (Maeda et al. 1984), which might suggest that it could also act as an antifeedant, thus inhibiting grazing by planktonic crustacea.

We conclude that the pennate diatom, *N. pungens* f. *multiseries*, was the major source of the domoic acid contained in the digestive glands of toxic mussels from the Cardigan Bay region of P.E.I. in late 1987 and 1988. In view of the global distribution of *N. pungens*, and the strong possibility that there may be other microalgal sources of domoic acid, contamination of shellfish by this toxin could be widespread. It is therefore important to understand the ecology of this organism, as well as the production and fate of domoic acid in the marine environment.

Acknowledgements

We acknowledge the cooperation of our colleagues at the Department of Fisheries and Oceans: R. Addison, M. Bewers, N. Crewe, M. DeRepentigny, P. Dickie, J. Stewart, and P. Vass (Bedford Institute of Oceanography, Dartmouth, N.S.); R. Drinnan, and D. Scarratt (Halifax, N.S.); J. Martin (St. Andrews, N.B.); D. Richard (Black's Harbour, N.B.); R. Angus, T. Sephton, and J. Worms (Moncton, N.B.); and L. Lea (Charlottetown, P.E.I.). We also acknolewdge the contributions of V. Adams, D. Beattie, and D. MacPhee (University of Prince Edward Island); T. Bernard, and I. Judson (Prince Edward Island Department of Fisheries); B. Marcotte (Department of Marine Resources, ME); and C. Carver and A. Mallet. We also thank our National Research Council colleagues for their invaluable assistance: R. Boyd, C. Craft, E. Dyer, D. Embree, M. Falk, M. Flack, C. Gillis, M. Greenwell, W. Hardstaff, F. Isaacs, W. Jamieson, D. Krailo, P. Leblanc, N. Lewis, G. McCully, M. McInerney-Northcott, J. McLachlan, G. McNeil, J. Milley, D. Moore, D. O'Neil, P. Seto, D. Tappen, A. R. Taylor, P. Thibault, and J. van Ingen. The active cooperation of eastern P.E.I. mussel growers (Atlantic Mussel Growers, Ltd. and North Ocean Enterprises, Ltd.) is greatly appreciated.

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Survival, Ocean Growth, and Ocean Distribution of Differentially Timed Releases of Hatchery Coho Salmon (*Oncorhynchus kisutch*)[1]

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Mathews, S. B., and Y. Ishida. 1989. Survival, ocean growth and ocean distribution of differentially timed releases of hatchery coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. 46: 1216–1226.

Estimates of growth rates in early ocean life and length frequency distributions at time of release for adult survivors were inferred from scale measurements for lots of coho salmon (*Oncorhynchus kisutch*) released at several dates from a Columbia River hatchery (Big Creek) and a southern Oregon hatchery (Coos Bay). Analysis of these measurements failed to support either of two literature-suggested hypotheses for the variability of marine survival of hatchery coho by date of release: (1) intraseasonal variability of food supply during early marine life; and (2) intraseasonally improving ability of smaller individuals within release lots to convert to salt water. Marine survival improved substantially with date of release at both hatcheries. However, fish released earliest, which survived relatively poorly, grew as fast in early marine life as fish released later, which survived better. Thus, poor survival of the early released fish did not appear related to lack of food at early ocean life. For the Big Creek hatchery, no significant differences were seen between the mean lengths at date of release and the mean release-date lengths of adult survivors back calculated from their scales, for lots released on May 5, June 3, and July 2. Apparently, small and large fish of each lot survived with equal probability. For the Coos Bay hatchery, the mean release-date lengths of adult survivors were significantly larger than the mean lengths of all fish released, for each of six lots released between early June and late July. Thus, large fish apparently survived better than small ones within each of these six lots. But there was no seasonal trend in survivability of small fish relative to large ones, contrary to hypothesis (2)

On a obtenu des estimations des taux de croissance au début de la vie océanique et de la distribution des longueurs au moment de la libération des survivants adultes, à partir des résultats de scalimétrie effectuée sur des saumons cohos (*Oncorhynchus kisutch*) répartis en lots et libérés à des dates différentes d'une écloserie du fleuve Columbia (Big Creek) et d'une autre dans le sud de l'Orégon (Coos Bay). L'analyse de ces mesures n'a permis de confirmer ni l'une ni l'autre des deux hypothèses formulées dans la documentation et portant sur la variabilité de la survie en milieu marin des saumons cohos produits en écloserie, selon la date de libération : 1) variabilité intrasaisonnière de la quantité de nourriture disponible au début de la vie océanique; et 2) amélioration intrasaisonnière de la capacité des petits individus libérés à s'adapter à l'eau salée. La survie en mer s'est améliorée sensiblement avec la date de libération des poissons des deux écloseries. Cependant, les saumons libérés en premier, et dont le taux de survie a été faible, se sont développés aussi rapidement, au début de leur vie océanique, que les individus libérés plus tard, qui ont survécu en plus grand nombre. En conséquence, le faible taux de survie des poissons libérés en premier ne semble pas lié au manque de nourriture pendant la période initiale de vie océanique. On n'a relevé aucune différence significative à l'écloserie de Big Creek entre les longueurs moyennes à la date de libération des individus et celles des survivants adultes, obtenues par rétrocalcul au moyen des écailles, pour des lots libérés le 5 mai, le 3 juin et le 2 juillet. Apparemment, la probabilité de survie des petits et des gros saumons de chaque lot était équivalente. Dans le cas de l'écloserie de Coos Bay, les longueurs moyennes à la libération des survivants adultes étaient nettement supérieures à celles de tous les poissons libérés, pour chacun de six lots de poissons dont la libération s'est échelonnée entre le début de juin et la fin de juillet. Il semble donc que les gros poissons aient un taux de survie plus élevé que les petits individus dans chacun de ces six lots. Cependant, contrairement à l'hypothèse (2), on n'a pas relevé de tendance saisonnière dans la capacité de survie des petits poissons comparativement aux saumons de grande taille.

Received January 15, 1988
Accepted February 23, 1989
(J9544)

Reçu le 15 janvier 1988
Accepté le 23 février 1989

Of all readily controllable conditions for rearing or releasing hatchery coho salmon (*Oncorhynchus kisutch*), date of release is perhaps the one to which marine survival responds the most and with the greatest consistency (S. B. Mathews, unpubl. data; Mathews and Buckley 1976; Novotny 1980; Bilton et al. 1982; Gowan and McNeil 1984; Bilton et al. 1984). Several-fold differences in survival usually occur within a series of sequential experimental releases during spring and summer, one to several months apart. When both individual size and date of release are jointly controlled, release size has less effect on survival than release date (S. B. Mathews, unpubl. data; Bilton et al. 1982; Bilton et al. 1984).

Why survival (mortality) should vary so much for fish released at similar size a month or two apart is not well under-

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stood. To gain the fullest survival benefits from optimal release timing, we need to understand how or which of the major mortality factors — predation, starvation, disease, or inability to adapt to salt water — are affected singly or in combination by release timing. It is, of course, difficult to assess relative impacts of these four factors on cohorts of salmon after release, but analysis of ocean growth, total ocean mortality, and ocean distribution of differentially-timed coded-wire tagged (CWT) release lots may offer clues.

Two hypotheses for the wide range in survival by date of release were suggested in recent literature: (1) intraseasonal variability of early ocean-life food supply for the coho, and (2) intraseasonal variability of physiological readiness of outmigrant coho to adapt to salt water. The food supply hypothesis is stated by Bilton et al. (1982):

> "Optimum conditions of time and size at release may be those providing for entry of juveniles into the nearshore and coastal ecosystems at a time and size when maximum advantage can be obtained from the available food supply in terms of growth and survival.... Conditions that maximize the food supply, such as onset of spring plankton pulse, in turn may vary geographically and temporally in relation to oceanographic events."

Simply put, a coho released too early might find too sparse a food supply for optimum growth and survival.

Two recent papers are pertinent to the hypothesis of intraseasonally varying physiological readiness. Zaugg (1982) showed that coho salmon, when held after their normal release period in May until June or July, initially lost some measures of their enzyme characteristics associated with smoltification, but regained these rapidly during outmigration; Zaugg stated that this ability could contribute to their better survival. Mahnken et al. (1982) summarized evidence that smoltification readiness influences survival and is controlled by photoperiod. They pointed out that the best release time from CWT experiments tended to be mid-June, the time of longest daylight, and that physiological conditions associated with smoltification could be manipulated by artificially regulating photoperiod. Mahnken et al. further suggested that the date-of-release survival mechanism could be size dependent. They found that within experimental lots transferred from the hatchery to salt-water holding pens at various times, the minimum size for adaptation to salt water tended to decrease over time through the spring until summer solstice and to increase thereafter.

In the present paper, we investigate these two hypotheses using measurements from scales sampled during two recent hatchery experiments in which various lots of CWT coho were released sequentially over spring and early summer. Scales were sampled from these cohorts at time of release and at adult return. These two experiments, like the ones referred to above, were to determine the extent to which postrelease survival depends upon date of release. Scale measurement analysis offers a way to search for biological mechanisms explaining the high variability in survival among releases made on different dates.

Scale measurements are commonly used for estimating body growth rates and inferring body sizes at ages younger than age at sampling. To investigate the ocean food supply hypothesis we estimated body sizes at time of formation of the (single) ocean annulus, for CWT adult returns from lots released at various dates. Body growth rates between time of release and time of ocean annulus formation were compared. To investigate the other hypothesis we back calculated the body lengths at the time of release from the scales of CWT adults returning to the hatcheries. Differences between mean release size of surviving adults and mean size from samples taken at time of release were compared for lots released at various dates. If marine survival depends on size at release such differences should be significantly greater than zero. If marine survival depends upon intraseasonal adaptability of the smaller individuals in a release lot to salt water, which may be photoperiod controlled, then the differences between back-calculated mean length and mean length of a lot at release should decline as release date approaches summer solstice and/or be correlated with total survival.

Methods

One of the experiments for which we conducted scale analysis involved CWT lots of 1979 year class coho released at age 1+ on three dates during 1981 from Big Creek hatchery (Fig. 1). This is a standard salmon hatchery on a tributary entering the Columbia River about 27 miles upstream from the ocean. The Big Creek hatchery is operated by the Oregon Department of Fish and Wildlife (ODFW) to enhance public fisheries for coho and chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Oncorhynchus mykiss*) in the Columbia River and adjacent coastal regions.

The other experiment involved CWT lots of 1982 year class coho released at age 1+ on five dates in 1984 and one 1983 year class lot released at age 0+ on a sixth date in 1984 from a hatchery on Coos Bay (southern Oregon coast; Fig. 1). The Coos Bay hatchery is owned by a private "ocean ranching" firm, Anadromous Inc., headquartered in Corvallis, Oregon, and releases both coho and chinook salmon to sea. Under Oregon law, such a privately owned hatchery must enhance the public fisheries, but those fish that escape the public fisheries to return to the release site may be sold by the firm for profit.

The Coos Bay facility should perhaps not be termed a hatchery, per se. It is actually a coastal release and adult trapping site for coho (and chinook) hatched and reared at inland sites. At Coos Bay saltwater is pumped through raceway ponds. Smolts are transported by truck to this facility from the inland sites and held there in almost full-strength saltwater (>30‰) for about 10 d for acclimation and return site imprinting, before release to sea.

The Big Creek experiment was designed by ODFW to determine when coho should be released to achieve maximum postrelease survival. The Coos Bay experiment was undertaken by Anadromous, Inc. to determine how release date affects profit. Profit would depend upon survival, of course, but also on average adult size and ocean (public fishery) catch rate.

Big Creek Releases and Scale Sampling

Personnel of ODFW raised the 1979 brood experimental lots, wire-tagged them, sampled for mean length and weight, and enumerated the outmigrants (A. R. Hemmingsen, Oregon Department of Fish and Wildlife, Corvallis, OR, pers. comm.). Additionally, samples were taken by ODFW at 2-wk intervals beginning in March 1981 to monitor plasma thyroxine concentrations and gill (Na+K)-ATPase activity as described by Ewing et al. (1985); these are commonly considered physiological indices of smoltification. Lots were released May 5, June 3, and July 2, 1981 (Table 1). During rearing, mean size was monitored and feeding rates among lots were adjusted in

FIG. 1. Locations of four lower Columbia River hatcheries at which date-of-release CWT experiments were conducted with 1977–79 year classes, and the Coos Bay hatchery.

TABLE 1. Summary of release, catch, and escapement information for three 1979 year class CWT experimental lots released in 1981 from Big Creek hatchery, OR, at approximately 1-mo intervals.

	Lot 1	Lot 2	Lot 3
Release date	5/5/81	6/3/81	7/2/81
No. released with CWT	54 038	55 711	54 374
No. sampled for release size	738	746	817
Mean release length (cm)	13.4	13.6	13.5
Standard deviation release length (cm)	0.91	0.96	1.08
Mean release weight (g)	23.5	24.5	23.7
Estimated 1982 catch	482	1786	1018
Estimated 1982 escapement	147	592	486
1982 Catch plus escapement	629	2378	1504
Survival rate excluding jacks (%)	1.16	4.27	2.77
Percent of 1982 catch by region:			
California coast	10.6	9.9	6.5
Oregon coast	58.7	66.0	70.3
Columbia River	11.0	12.4	11.4
Washington coast	14.7	9.7	10.6
Puget Sound	0.6	0.0	0.0
British Columbia coast	3.5	2.0	1.2
Unspecified river	0.8	0.0	0.0

an attempt to equalize mean release size among the three lots; this was to minimize the confounding of release size and release date effects on survival. Release lengths (fork) averaged 134, 136, and 135 mm for the three dates, respectively. There were two replicate raceway ponds of separately coded wire-tagged fish for each release date, but survival and geographical distribution of catch were so similar for replicates of each of the three release dates that the data were combined for each pair of replicates in Table 1 and in further reported results.

For a scale sample at release, a single dip-net sample of $n = 34$ smolts was taken by the hatchery manager from one of the May release ponds on April 30, 1981. These fish were frozen for 6 mo before measuring and removing scales. After thawing, we measured each fish (fork length to nearest millimetre) and took samples of up to 10 scales from the left side of the fish in the preferred region according to Clutter and Whitesal (1956). The preferred region is two to four rows above the lateral line on a 45° diagonal line between the posterior insertion of the dorsal fin and the anterior insertion of the anal fin. Four scales that were neither abnormally shaped nor showed a regenerated nucleus under 18× binocular scope viewing were taken from each fish, mounted between two glass microscope slides, and read at 68× magnification with a Vantage III microform reader. For each fish, the scale with the largest radius (center of focus to the outer, forward edge) of these four was measured. Because the fork lengths were measured from fish frozen for 6 mo, such lengths had to be corrected (upwards) for shrinkage due to

TABLE 2. Summary of release, catch, and escapement information for five 1982 year class and one 1983 year class CWT experimental lots released into Coos Bay, OR, in 1984 at approximately 10-d intervals.

	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6[a]
Release date	6/8/84	6/18–19/84	6/26/84	7/9/84	7/18/84	7/18/84
No. released with CWT	13 770	22 008	23 305	18 925	19 396	27 512
No. sampled for release size	90	74	77	79	83	79
Mean release length (cm)	15.3	15.1	15.3	15.6	15.8	17.5
Standard deviation release length (cm)	0.94	0.78	0.74	0.88	0.79	1.12
Mean release weight (g)	38.8	35.0	39.8	42.0	44.5	58.2
Estimated 1985 catch	36	31	161	89	167	445
Estimated 1985 escapement	95	210	713	560	831	1727
1985 catch plus escapement	131	241	874	649	998	2172
Survival rate excluding jacks (%)	0.95	1.09	3.75	3.43	5.14	7.89
Percent of 1985 catch by region:						
California coast	0.0	19.4	0.0	0.0	1.8	1.6
Oregon coast	22.2	58.0	57.3	70.0	84.3	84.0
Washington coast	22.2	22.5	23.1	15.5	13.8	9.2
British Columbia coast	55.5	0.0	19.5	14.4	0.0	5.1

[a]0+ age coho; all other lots were released as 1+ aged yearlings.

freezing. We determined the shrinkage correction factor (corrected length equals frozen length multiplied by 1.0559) from an independent second sample (from the Green River hatchery, Puget Sound) of 32 coho of a length range similar to the original Big Creek sample. Fork lengths from this second sample were measured before and after the fish were frozen for 6 mo. The after-freezing lengths averaged 5.29% shorter than the before-freezing lengths; the difference was highly significant with a paired sample t-test ($p<0.01$), and the relative shrinkage factor was independent of length ($0.20<p<0.50$ of the null hypothesis $\beta = 0$).

The adults returning to Big Creek hatchery in 1982 were sampled for CWT by ODFW. We took scales from a random sample of the adipose fin-clipped/wire-tagged adults during the escapement sampling process. We took two scales from the preferred region of each side of each fish (total of four from each fish). Scales were selected that appeared normal in size and shape to the naked eye. These were mounted on gummed cards from which 0.020-inch (0.508-mm) thick acetate impressions were later made using a scale press for 3 min at 4200 psi and 117°C temperature. Adult scales were read at 104× magnification with a microcomputer-based digitizer at the University of Washington Fisheries Research Institute (FRI).

Coos Bay Releases and Scale Sampling

Anadromous Inc. raised the fish, tagged them, enumerated the releases and sampled for mean size at release. Data for each of the six release-date lots (Table 2) were provided (R. Gowan, Anadromous Inc., Corvallis, OR, pers. comm.). The six experimental lots were released at approximately 10-d intervals from early June to late July 1984. The five release dates for the age 1+ lots were June 8, June 18–19, June 26, July 9, and July 18. The 0+ age lot was released July 31. There was no special effort to control mean size at release, as opposed to the Big Creek experiment, but the mean lengths (fork) among the five 1+ age lots were reasonably close: 153, 151, 153, 156, and 158 mm by date of release, respectively. Fish in the 0+ lot averaged considerably larger than the other five lots (175 mm). The larger size of this latter lot was due not only to its late release date, but also its unique composition. This lot was constituted early in rearing by size grading for the largest, probably fastest growing individuals from a larger population, which is a necessary fish cultural procedure to achieve smoltification in a high proportion of individuals at age 0+ (R. Gowan, pers. comm.). Due to the unique characteristics of the 0+ age lot, it was excluded in some of the statistical comparisons below.

Anadromous Inc., provided us with scales taken at release and scales taken from adults of each of the five age 1+ lots and the age 0+ lot. On each date of release, they took random samples ranging from $n = 71$ to $n = 90$ for size and scales. They also took scales from all adults returning in 1985 with an excised adipose fin, the external mark given at time of tagging to indicate the presence of the internal wire tag. We read adult scales from random samples of hatchery returns of each of the six date-of-release groups; the number of fish in the samples ranged from 66 to 115 adults per release group. According to R. Gowan (pers. comm.) their scale collection procedure for both release and adult samples was to select, with the unaided eye, four well formed scales from the preferred region of each side of each fish. These were placed on gummed cards from which acetate impressions were made. Anadromous Inc. staff measured the scale radii of the release samples from the largest well formed scale of each sampled fish under 45× magnification and provided us with these measurements as well as their scale collections. We randomly spot checked their measurements to confirm that uniformity existed between their methods and ours. We read the adult scales from their acetate impressions at 104× magnification with FRI's digitizer, selecting from each fish the scale with the largest freshwater zone for measurement.

Sampling of Adults for Coded-Wire Tags

Our estimate of the postrelease survival rate for a CWT lot is the estimated adult (3-yr old) catch by all fisheries and the spawning escapement divided by the number released. (We excluded estimates of catch and escapement of jacks, precocious 2-yr old males, because they were less than 2% of the total catch and escapement of any experimental lot and are not an economically important component of coho production.) In the present experiments wire tags were recovered and decoded by ODFW (Big Creek) and Anadromous Inc. (Coos Bay). Catch

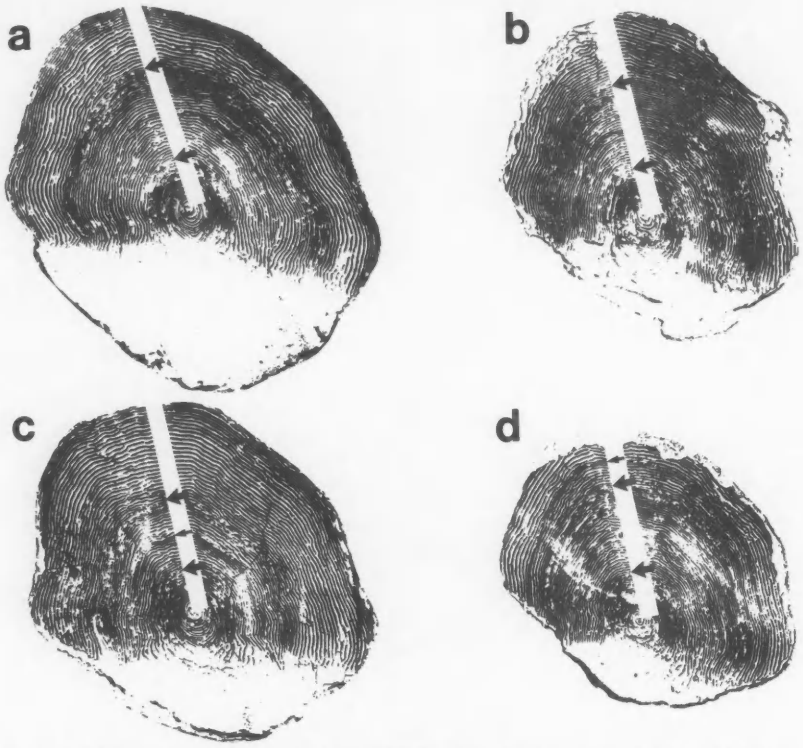

FIG. 2. Adult coho salmon scales at 104× magnification. Inner large arrows mark the transition between freshwater and saltwater growth, assumed to be the outer scale edge at time of release. Outer large arrows mark the ocean annulus. Small arrows in c and d show additional scale checks of unknown cause that occurred in about 4% of the sampled adult scales.

sampling for adipose-clipped/wire-tagged salmon at rates generally exceeding 10% is done by trained agency observers in all salmon fisheries of California, Oregon, Washington, British Columbia, and Alaska. Raw CWT data to estimate catches by region, including observed numbers by tag code, fishery, time period, and sampling rates by fishery and time period, are contained in a coast-wide CWT database maintained by the Washington Department of Fisheries (WDF) on a main frame computer system at the University of Washington. Software developed by R. O'Conner (WDF, Olympia, WA, pers. comm.) was used to access these and all other CWT-based data presented in this paper (WDF program SCANTG6).

Catch estimates were needed not only to estimate total survival rate of each release lot, but also to determine whether or not fish released on different dates were distributed similarly at sea. If there were major differences in latitudinal centers of distributions at sea among release lots, this could result in differential growth rates and confound comparisons of growth rates by date of release. It is assumed that if ocean catch distributions were similar among release lots, then early ocean migration patterns were also similar.

Scale Analyses

All scale measurements were taken from the focus toward the anterior edge along the longest (horizontal) axis (Fig. 2). For the release-sample scales, the distance from the focus to the outer edge of the scale was measured. For the adult scales, three measurements were taken: focus to the outer scale edge of the freshwater zone, focus to the saltwater annulus, and focus to the other scale edge, if the outer edge was not badly broken or resorbed.

The transition point between freshwater and saltwater zones, which we assumed corresponded to the edge of the scale at the date of release, is reasonably distinct for most adult coho scales (Fig. 2). It is commonly considered the point beyond which the circuli abruptly increase in thickness and spacing or that point from which the circuli on the scale's posterior portion start to branch outward toward the edge of the scale (K. W. Myers, University of Washington, Fisheries Research Institute, Seattle, WA, pers. comm.).

For about 92% of the Big Creek adults, the ocean annulus was readily discernible as the outer edge of a narrow region of several tightly spaced circuli in the saltwater zone (Fig. 2A). For about 4% of these adults, an area of merged circuli was considered to demarcate the annulus (Fig. 2B), and for the remaining 4% there were two or more regions interpretable as the ocean annulus according to checking, merging, or close spacing of circuli (Figs. 2C and D). For the latter cases, circuli were counted outward from the end of the freshwater zone to each potential annulus, and the annulus was chosen by referring to the number of circuli in this same scale region of fish considered to have an unambiguous scale location of annulus for-

mation. The Coos Bay samples contained a lesser proportion of ambiguous scales than the Big Creek samples.

We also made several different circulus counts and spacing measurements in the innermost portion of the ocean growth zone to try to find scale characteristics that might correlate with early ocean growth rate. These data are difficult to interpret, since it is undetermined whether circulus spacing distance in this region is positively or negatively correlated with growth of scales or other hard body parts (Major and Craddock 1962; Bilton and Robins 1971; Bilton 1975; Neilson et al. 1985). In our opinion, the best scale characteristic for relatively unambiguous comparison of ocean growth rates is the zone width between end of freshwater growth and the ocean annulus. Relating this measurement to body length provides an estimate of the average growth rate from the time of release to the time in late winter or early spring when the annulus is formed. The precise time of annulus formation is unknown — we assume February 1 when central Oregon sea surface temperatures are coldest (National Meteorological Center 1985) — but should be similar for different groups of fish with similar ocean distribution.

We did not have coho samples from mid-ocean life to include in establishing a relationship between body length and scale radius. However, to estimate length at the time of ocean annulus formation least square linear regression relationships (one for the Big Creek data and another for the Coos Bay data) were computed from body length measurements and scale radii of both the release and adult samples.

To back calculate lengths at release from the radii of the freshwater zones of adult scales, we utilized linear regression equations between scale radius (X) and body length (Y) from the release samples only. (As discussed below, this was done because body length/scale radius relationships from the release samples only appeared by eye to differ from the linear relationships fitted to both the release and adult samples.) To estimate the variance of the length distribution of adult survivors at their time of release (σ_y^2), the following approximation procedure was used.

$$S_y^2 = b^2 S_x^2 + S^2_{y/x}$$

where Y = release length in centimetres; X = scale radius in millimetres; S_y^2 = an estimate of σ_y^2; b = least squares estimate of the slope of the body length/scale radius relationship from the release sample; S_x^2 = variance of the freshwater scale radius in millimetres of the adult sample; and $S^2_{y/x}$ = the estimate of the about regression variance from the release sample. This equation follows directly from the linear regression model: $Y_i = \alpha + \beta X_i + E_i$, where $E_i \sim N(o,\sigma_{y2/x})$; then $\sigma^2_y = \beta^2\sigma^2_x + \sigma^2_{y/x}$.

Because of statistical errors in estimating β from a smolt sample, a slight bias would be expected from this method of estimating σ^2_y. However, we confirmed that results of a t-test comparing mean length at release with mean back-calculated release length were little affected by values of b ranging between $b \pm 2\sqrt{\text{var}(b)}$.

Mean fork lengths (centimetres) were converted to mean body weights (grams) with either of two large sample length/weight relationships. The one used for estimating mean weight (W) at ocean annulus formation from mean length (L) estimates was from coho lengths and weights sampled over several years and all months from the year-round Puget Sound sport fishery (Richard Geist, WDF, Olympia, WA, pers. comm.). The equation for estimating mean adult weight from mean length, provided by Anadromous Inc. (Mary McGowan, Coos Bay, OR, pers. comm.), was derived from fork length and weight measurements from several thousand 1985 and 1986 returning adults.

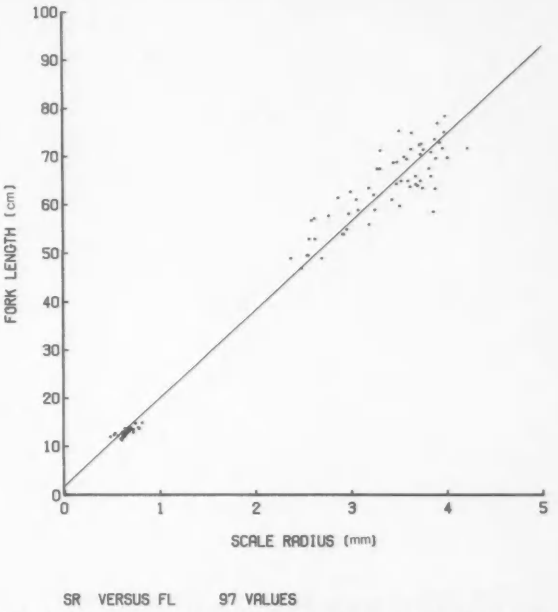

FIG. 3. Scattergram of fork length in centimetres (Y) and total scale radius in millimetres (X) for 34 outmigrants and 61 adults from the 1979 year class released at Big Creek hatchery in 1981. Fitted line: $Y = 18.250X + 1.643$.

$$W = 0.01507L^{2.940} \text{ Puget Sound}$$
$$W = 0.02582L^{2.825} \text{ Anadromous Inc.}$$

Average ocean growth rates for the various release lots were compared using estimates of the exponential growth coefficient, k, in the model $W = W_0e^{kt}$, or $k = \ln\left(\frac{W}{W_0}\right)\frac{1}{t}$, where W_0 = weight at some initial reference time and W = weight after t mo of growth. Mathews and Buckley (1976) showed that exponential growth might be a suitable model for Puget Sound coho during this part of ocean life.

Results

Effects of Release Date on Survival

Postrelease survival rates varied substantially among release dates; earliest releases tended to survive at lowest rates. For the Big Creek experiment, survival rates were 1.16, 4.27, and 2.77% for the May, June, and July release lots, respectively (Table 1). Thus, the middle date in that series was optimal. For Coos Bay, the survival rates by order of release date were 0.95, 1.09, 3.75, 3.43, 5.14, and 7.89%, indicating an increasing trend throughout the period (Table 2). The latest released and best surviving lot was the 0+ age lot, but these fish averaged almost 50% heavier at release than the mean release weight of the other five age 1+ lots (Table 1).

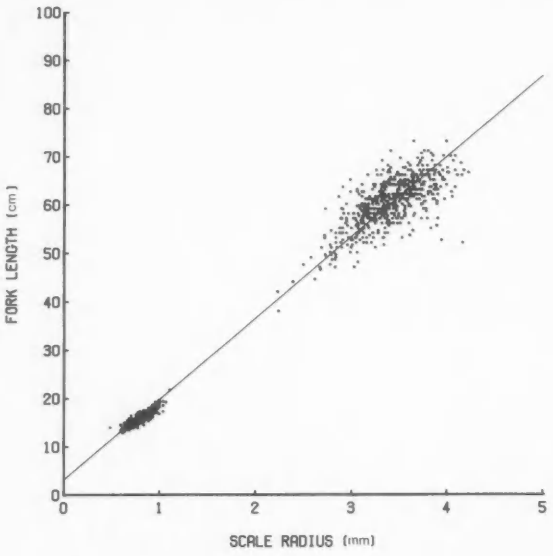

FIG. 4. Scattergram of fork length in centimetres (Y) and total scale radius in millimetres (X) for 479 outmigrants and 592 adults from the 1982 and 1983 year classes released at Coos Bay in 1984. Fitted line: $Y = 16.740X + 2.910$.

TABLE 3. Linear regression equations between scale radius millimetres (X) and fork length in centimetres (Y) for the Big Creek smolt sample and the smolt samples of each of the six experimental groups released in Coos Bay.

Experiment	Date of sampling	n	Regression equation	r^2	$S^2_{y/x}$
Big Creek	4/30/81	34	$Y = 8.770X + 7.188$	0.58	0.27
Coos Bay	6/8/84	90	$Y = 9.796X + 7.987$	0.65	0.31
Coos Bay	6/18–19/84	71	$Y = 11.354X + 6.649$	0.57	0.25
Coos Bay	6/26/84	77	$Y = 9.162X + 8.529$	0.44	0.31
Coos Bay	7/9/84	79	$Y = 9.548X + 8.144$	0.51	0.39
Coos Bay	7/18/84	83	$Y = 7.961X + 9.440$	0.59	0.26
Coos Bay	7/31/84	79	$Y = 13.305X + 5.447$	0.74	0.39

Effects of Release Date on Ocean Distribution

Release date did not appear to affect the latitudinal center of the catch distribution but it did affect the range. With one exception the majority of the catch of each lot in both experiments was taken off Oregon (Tables 1 and 2). The exception was the earliest released Coos Bay lot; of the estimated total catch from this lot (36 fish), 55.5% was from British Columbia. However, this apparent exception may be an anomaly of small sample size and low sampling rate (the estimated British Columbia catch of 20 fish was derived from an observed sample of only 2 CWT fish expanded by a factor of 10 in a fishery for which the sampling rate was about 10%). In both experiments, there was a trend towards geographical compression of the catch distribution with later release date. The proportions caught off Oregon tended to increase by date of release, with a corresponding decrease in proportions caught at the extremes of the range (Tables 1 and 2); these trends were significant ($p < 0.001$) for each experiment according to chi-square contingency table analysis.

Body Length/Scale Radius Relationships

Scattergrams between scale radius (Y) and fork length (X), including data from both the smolt samples and the adult samples, indicated that linear regression relationships were well suited for back calculating length at ocean annulus formation from the widths of adult scales at the point of ocean annulus (Figs. 3 and 4). Separate least square regression equations were estimated for each experiment:

$$Y = 18.250X + 1.643$$

Big Creek $r^2 = .977$

$$n = 95$$

$$Y = 16.740X + 2.910$$

Coos Bay $r^2 = .942$

$$n = 1071$$

Visual inspection of these scattergrams lead us to conclude that the body length/scale radius relationships for the smolts alone would probably be better defined with linear relationships of lesser slopes than the ones fitted to both smolt and adult samples; this is most evident for the Coos Bay experiment because of larger sample size (Fig. 4). Consequently, there would probably be less bias in back calculating length at release from the release check radii of adult scales using the body length/scale radius regression equation for smolts only rather than the regression equations fitted to both smolt and adult scales. For the Coos Bay experiment scales were sampled from a substantial number of smolts of each release lot ($n = 70$, 71, 77, 79, 79, 83 individuals per lot). Due to sufficient sample sizes and because the body length/scale radius relationship probably changes with time due to a rapid rate of elongation of the body in the caudal region during smoltification (Winans 1985), we estimated six separate regression equations for the Coos Bay lots (Table 3) and used these separately for back calculating release lengths. Separate equations for each release date would also have been preferable for the Big Creek analysis, but scales were available only from smolts of the first release date (Table 3).

Effects of Release Date on Size at Ocean Annulus Formation

Except for the 0+ age lot from Coos Bay, mean length and weight at ocean annulus formation declined with date of release (Table 4). In both experiments the differences were significant (according to analysis of variance of lengths) and substantial, particularly in terms of weight. Big Creek coho released earliest (May 5) averaged 39% heavier at the time of ocean annulus formation than fish released latest (July 2). Of the five 1+ age lots from Coos Bay the mean weight at annulus formation of the earliest released lot (June 8) was 29% greater than the mean of the latest (July 18).

Effects of Date of Release on Adult Size

Except for the 0+ age lot from the Coos Bay hatchery, adult size tended to decrease with date of release; but the relative differences among mean weights at adulthood were less than at time of ocean annulus formation (Table 5). Adults from the earliest release at Big Creek averaged 13% heavier than those from the latest release. Adults from the earliest release at Coos Bay also averaged 13% heavier than those from the latest released 1+ age lot.

TABLE 4. Estimated lengths and weights at time of ocean annulus formation for adult coho salmon returning to Big Creek and Coos Bay hatcheries from various release dates.

Big Creek

	Date of release		
	5/5/81	6/3/81	7/2/81
No. of readable scales	42	120	67
Scale radius to ocean annulus (mm)			
Mean[a]	2.19	2.10	1.95
Standard deviation	0.21	0.23	0.22
Mean fork length at ocean annulus (cm)	41.61	39.97	37.23
Mean weight at ocean annulus (g)	868	771	622

Coos Bay

	Date of release					
	6/8/84	6/18–19/84	6/26/84	7/9/84	7/18/84	7/31/84
No. of readable scale	70	71	77	79	83	79
Scale radius to ocean annulus (mm)						
Mean[b]	2.10	2.02	1.99	1.94	1.91	1.97
Standard deviation	0.16	0.13	0.12	0.13	0.14	0.14
Mean fork length at ocean annulus (cm)	38.06	36.72	36.22	35.38	34.88	35.88
Mean weight at ocean annulus (g)	668	601	577	539	517	562

[a]Means significantly different, $F = 21.00$, $p < .001$.
[b]Means significantly different, $F = 25.90$, $p < .001$.

TABLE 5. Estimated lengths and weights of adult coho salmon returning to Big Creek and Coos Bay hatcheries from various release dates.

Big Creek

	Date of release		
	5/5/81	6/3/81	7/2/81
No. of adults measured	42	125	171
Fork length (cm)			
Mean[a]	66.70	65.20	63.80
Standard deviation	5.26	5.11	5.08
Mean weight (g)	3,674	3,445	3,240

Coos Bay

	Date of release					
	6/8/84	6/18–19/84	6/26/84	7/9/84	7/18/84	7/31/84
No. of adults measured	66	100	100	115	111	100
Fork length (cm)						
Mean[b]	61.90	60.30	61.00	60.00	59.30	60.90
Standard deviation	4.97	4.80	4.77	4.95	4.81	5.01
Mean weight (g)	2,975	2,763	2,854	2,724	2,635	2,841

[a]Means significantly different, $F = 4.18$, $p < .05$.
[b]Means significantly different, $F = 4.04$, $p < .01$.

Effects of Date of Release on Ocean Growth Rates

Exponential growth coefficients (k) for the time between release date and date of ocean annulus formation increased somewhat by date of release for the Big Creek lots (Table 6). But this trend may be (as later discussed) an artifact of an imperfect growth model and not evidence that fish released in May (which survived the poorest) grew slower for their initial time at sea than fish released in June or July. Estimates of k between release date and ocean annulus date were virtually the same for all six of the Coos Bay release lots (Table 6).

For both experiments, the estimates of k for the period between annulus formation and adulthood tended to increase by date of release (table 6). Such trends reflect the apparent tendency for the smallest (earliest released) fish at the time of ocean annulus formation to "catch up" in growth by adulthood, i.e. narrow the gap in size differential.

Effects of Size at Release on Survival

Mean release size was not a treatment variable in either experiment. However, the difference between the mean of the back-calculated release lengths of a sample of adults from a lot and the mean length of this lot at the time of release is one measure of the extent to which survival within a release lot

TABLE 6. Exponential ocean growth coefficients (k per month) for lots of coho released at various dates from Big Creek and Coos Bay hatcheries.

Big Creek

	Date of release		
	5/5/81	6/3/81	7/2/81
k from time of release to time of ocean annulus formation	0.40 (t=8.8)	0.43 (t=7.9)	0.47 (t=6.9)
k from time of ocean annulus formation to end of ocean life	0.19 (t=7.5)	0.20 (t=7.5)	0.22 (t=7.5)

Coos Bay

	Date of release					
	6/8/84	6/18–19/84	6/26/84	7/9/84	7/18/84	7/31/84
k from time of release to time of ocean annulus formation	0.37 (t=7.7)	0.38 (t=7.4)	0.38 (t=7.1)	0.38 (t=6.7)	0.38 (t=6.4)	0.38 (t=6.0)
k from time of ocean annulus formation to end of ocean life	0.20 (t=7.5)	0.20 (t=7.5)	0.21 (t=7.5)	0.22 (t=7.5)	0.22 (t=7.5)	0.22 (t=7.5)

TABLE 7. Comparison of mean lengths at release and mean back-calculated release lengths of adult survivors for lots released on three dates from Big Creek hatchery.

	Date of release		
	5/5/81	6/3/81	7/2/81
Release sample			
No. measured	738	746	817
Mean length (cm)[a]	13.40	13.60	13.50
Standard deviation length (cm)	0.91	0.96	1.08
Mean weight (g)	23.5	24.5	23.7
Adult scale sample			
Sample No. with scales read	42	120	67
Freshwater zone scale radius			
Mean (mm)[b]	0.72	0.74	0.73
Standard deviation (mm)	0.08	0.08	0.08
Back-calculated release length			
Mean (cm)	13.50	13.68	13.59
Standard deviation (cm)	0.79	0.79	0.79
t-test for null hypothesis of equality between release length and back-calculated release length means	t=0.70 n.s.	t=0.82 n.s.	t=0.59 n.s.

[a]Means significantly different, $F=11.01$, $p<.001$.
[b]Means not significantly different, $F=1.05$.

depends upon size. If the probability of survival within a lot is independent of release size, these two means should be equal; or, if larger fish in a lot have a higher chance of surviving than smaller ones, then the mean of the back-calculated release lengths should exceed the mean length of the lot at release. The greater the difference in the two, the greater the dependency of survival on size at release.

For the Big Creek experiment, there was little evidence of size-dependent survival within any of the release lots. Mean lengths at release and mean back-calculated lengths of adult survivors were 13.40/13.50, 13.60/13.68, and 13.50/13.59 cm for the May 5, June 3, and July 2 release lots, respectively (Table 7). Although the back-calculated means were slightly larger in each case, the pairs of means did not differ significantly from one another for any of the three release dates (two group t-tests of equal means, Table 7).

For the Coos Bay experiment, the mean lengths at release of the adult survivors were significantly larger than the mean lengths at release for each of the six release lots (two group t-tests; $p<0.001$), suggesting greater marine mortality of fish smaller at release than larger ones (Table 8). There was no evidence of a time trend in the differences between mean back-calculated release length of adult survivors and the mean size at release, although such a trend would be expected if higher survival with later release was the result of progressively improved adaptability to saltwater of smaller fish with later release. These differences ranged from about 0.5 to 1.0 cm (Table 8), but were not significantly correlated with release date ($r=0.227$, $p>0.30$, $n=6$) or survival rate ($r=-0.325$, $p>0.25$, $n=6$).

Discussion

Results of the two date-of-release experiments augmented with scale analysis indicate several trends in common. However, the evidence that marine mortality was dependent on size at release differed between the two experiments.

The observations common to the two experiments were:
(1) Geographical range at sea according to distribution of fishery recoveries by region compresses with later release date.
(2) The earlier the release date the greater the size of the individual at ocean annulus formation.
(3) The earlier the release date the greater the adult size.
(4) The relative difference in size among date-of-release lots is greater at time of annulus formation than at end of ocean life.

Observation (1) is not surprising: the earlier the release date, the longer the time for ocean dispersal. For coho released into Puget Sound at varying dates between April and August 1, Mathews and Buckley (1976) showed that the later the release date, the greater the tendency to reside in Puget Sound the entire life rather than migrate to the open ocean. Coho released earlier by Bilton et al. (1984) tended to have a more extended range than those released later.

TABLE 8. Comparison of mean lengths at release and mean back-calculated release lengths of adult survivors for lots released on six dates from the Coos Bay hatchery.

	Date of release					
	6/8/84	6/18–19/84	6/26/84	7/9/84	7/18/84	7/31/84
Release sample						
No. measured	70	71	77	79	83	79
mean length (cm)[a]	15.26	15.05	15.29	15.62	15.79	17.47
Standard deviation length (cm)	0.94	0.78	0.74	0.88	0.79	1.12
Mean weight (g)	38.8	35.5	39.8	42.0	44.5	58.2
Adult sample						
Sample No. with scales read	66	100	100	115	111	100
Freshwater zone scale radius						
Mean (mm)[b]	0.80	0.83	0.84	0.87	0.89	0.95
Standard deviation (cm)	0.10	0.09	0.08	0.09	0.09	0.10
Back-calculated release length						
Mean (cm)	15.82	16.07	16.22	16.45	16.52	18.09
Standard deviation (mm)	1.12	1.14	0.92	1.06	0.87	1.47
Difference between back-calculated and release length means (cm)	0.56	1.01	0.93	0.83	0.73	0.62
t-test for null hypothesis of equality between release lengths and back-calculated release length means	$t=3.37$ $p<0.0001$	$t=6.47$ $p<0.0001$	$t=7.26$ $p<0.0001$	$t=5.73$ $p<0.0001$	$t=6.01$ $p<0.0001$	$t=3.11$ $p<0.0001$

[a]Means significantly different, $F=10.42$, $p<.001$.
[b]Means significantly different, $F=17.19$, $p<.001$.

According to observation (2) it is not likely that the relatively low survival of the earliest releases was related to suboptimal feeding conditions. The differences in weight at annulus formation between earliest and latest released lots were substantial in both experiments indicating good growth rates during the time between such release dates. The decline in the exponential growth coefficient for the earlier ocean life period by date of release in the Big Creek experiment may be due to sampling error or may be the artifact of an imperfect growth model, rather than indicative of slower early growth of early releases. If a linear model for growth in weight is assumed, the average monthly rate from release date to ocean annulus formation is actually greater for the May release lot than for the other two lots.

Observation (3), that adult size is less with later release, was also shown by Bilton et al. (1982), and Bilton et al. (1984) and S. B. Mathews, unpubl. data. The apparent reason is that growth rate at sea is constant and thus the longer a fish is at sea the greater its final size.

Observation (4), that range in mean size among release lots narrowed between time of annulus formation and end of ocean life, has no obvious explanation. Perhaps this is due to compensatory growth; fish smaller at ocean annulus formation grow faster than larger ones for the rest of their life. It could also be due to size-dependent fishing or natural mortality forces. Further speculation is not warranted.

That marine survival within release lots was independent of size at release was evident from the Big Creek results. Such results are consistent with several previous hatchery experiments in which effects of mean release size on survival were evaluated by producing sub-lots of different mean sizes by size grading at time of release from a larger lot (Hager and Noble 1976; Bilton et al. 1982; Bilton et al. 1984). In such experiments marine survival rate of tagged lots (excluding jack production) was affected relatively little by mean sub-lot size. Scale analysis results by Rankis (1987) from several Columbia River hatchery experiments evaluating rearing density consistently indicated minimal effect of individual size within release lots on postrelease survival probability. Survival of coho has often been found to increase with mean release size in a number of experiments where mean size per release lot is controlled during rearing by feeding ration, length of time reared, or rearing temperature (Wallis 1968; Johnson 1970; Senn and Noble 1968; C. W. Hopley, Washington Department of Fisheries, Olympia, Wash., pers. comm.; Fuss and Rasch 1981; Gowan 1988). Treatment factors that correlated with size rather than size per se may have caused the apparent size-related survival response in such experiments.

Conflictingly, the Coos Bay experiment offered evidence that larger fish in a release lot survived better than smaller ones (Table 8). At Coos Bay, coho are transferred directly to almost full strength saltwater after trucking them from the freshwater rearing site. In the Big Creek experiment and all other circumstances referred to above that showed minimal evidence for size-dependent postrelease survivability within release lots, the fish had the opportunity for choice in movement relative to salinity gradients encountered as they migrated to sea. The conflicting results from the Coos Bay experiment could be due to size-dependent stress from size-related variability in osmoregulatory ability in a forced, steep salinity gradient. The only other marking or tagging evidence for size-dependent, postrelease survivability within lots of commonly-reared coho of which we are aware is from another private ocean ranching facility on the Oregon coast that also introduces fish into saltwater ponds several days prior to ocean release (Stohr and Parker 1982; Wilson 1986). The latter operation differs from previously discussed experiments in that it releases coho at age 0+, not at age 1+, and these releases produce 2-yr old, not 3-yr old, adults.

In summary, the results of our scale analysis seem at odds with both of the earlier stated hypotheses for the variability in postrelease survival of coho salmon by date of release. There was little evidence that fish released earliest encountered poor feeding conditions or that survival of smaller fish was improved with date of release.

There is substantial and conflicting literature on the relationship between smoltification condition, as indexed by time varying physiological measurements, and optimum release date. This issue, which is beyond the scope of the present paper, is reviewed by Ewing et al. (1985). They found little relationship between two well studied smoltification indices (plasma thyroxine concentration and gill (Na+K)-ATPase activity) and optimum release date for several experiments involving both chinook and coho salmon, including the 1979 year class Big Creek experiment.

Clearly, more experimentation is needed to determine whether optimal release date is an interannually "moving target" dependent upon a variable receiving environment, a function of smolting response dependent upon an interannually invariant cue such as photoperiod, or perhaps a function of a smolting response that depends upon variable factors in the rearing environment such as water temperature. In spite of the number of date-of-release experiments, there has simply been insufficient geographical and interannual replication to answer such questions. Perhaps predation is a more likely time-varying mortality force. Most avian, mammal, and fish predators migrate on seasonal schedules, upon which are superimposed more or less random fluctuations in geographical distribution caused in response to fluctuations in prey distribution. Offshore release from a mobile net pen of coho salmon smolts prior to release on the Oregon coast by Anadromous Inc., and Oregon Aquafoods (another Oregon ocean ranching firm), which was intended to reduce inshore avian predation, substantially improved survival during 2 of 3 yr of controlled CWT experiments (Richard Severson, Oregon Aquafoods, Springfield, OR, pers. comm.).

It would be clearly unwise to attribute date-of-release survival variability to any single mortality category from the fragmentary evidence obtained this far. There are too many potential interactions; for example, relationships between incidence of disease in early ocean life and time-dependent physiological conditions associated with smoltification are virtually unstudied.

Acknowledgements

The cooperation of the Oregon Department of Fish and Wildlife and Anadromous Inc. is gratefully acknowledged, especially Al Hemmingsen (ODFW) and Ron Gowan (Anadromous Inc.). Katherine W. Myers of Fisheries Research Institute was helpful with questions on scale interpretation. Karen Halliday (formerly at FRI, now at National Marine Fisheries Service) helped in sampling and measuring Big Creek scales. This is an incomplete list of collaborators since many hatchery workers and wire-tag samplers assisted. Washington Sea Grant provided finanical support. The results described for the Big Creek hatchery experiment are part of a coordinated series of date-of-release experiments at four Columbia River hatcheries conducted by three public agencies, ODFW, WDF, and U.S. National Marine Fisheries Service.

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Spatial and Temporal Patterns of Hydrogen Peroxide in Lake Waters

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Depth profiles of H_2O_2 concentration were measured at three stations in Lake Erie, one in Lake Ontario and in Jacks Lake. Epilimnetic concentrations followed changes in solar radiation suggesting that the formation resulted from photochemical or perhaps photoautotrophic processes. Mid-day H_2O_2 concentrations of 100–200 nM were observed at all locations. Dark decay of H_2O_2, determined at several depths at two locations, followed apparent first order kinetics with half lives from 10–22 h. H_2O_2 may be useful as a tracer for short term mixing processes and as a powerful oxidizing agent can influence metal speciation, degradation of some organic pollutants, as well as the survival and behavior of organisms.

On a déterminé les profils de concentration de H_2O_2 à trois stations dans le lac Erié, une dans le lac Ontario et une dans le lac Jacks. La variation des concentrations épilimniques en fonction du rayonnement solaire indique que ce phénomène est le produit de processus photochimiques ou, peut-être, de la photo-synthèse. Des concentrations de H_2O_2 de 100 à 200 nM ont été relevées à midi à toutes les stations. La décomposition dans l'obscurité, observée à différentes profondeurs à deux endroits, a obéi à une réaction cinétique qui semble être du premier ordre avec des demi-vies biologiques de 10 à 22 h. H_2O_2 pourrait servir de traceur dans les processus de brassage à court terme et, à titre d'oxydant énergétique, il peut avoir un effet sur la différenciation des espèces métalliques, la dégradation de certains polluants organiques, ainsi que la survie et le comportement des organismes.

Received June 27, 1988
Accepted February 23, 1989
(J9790)

Reçu le 27 juin 1988
Accepté le 23 février 1989

Hydrogen peroxide (H_2O_2) has been measured in oceans (Zika et al. 1985a,b) and in fresh waters (Sinel'nikov 1971a,b; Sinel'nikov and Demina 1974; Sinel'nikov and Liberman 1974; Draper and Crosby 1981a,b; 1983a,b; Cooper and Zika 1983; Cooper et al. 1988, 1989; Klockow and Jacob 1986; Cooper and Lean 1989), but information on spatial and temporal patterns has never been obtained for lakes. Cooper and Lean (1989) measured diel changes in lake surface H_2O_2 concentrations that were an order of magnitude greater than that previously observed in surface oceans. In the following investigation depth profiles of H_2O_2 concentration were obtained in Lake Erie, Lake Ontario, and Jacks Lake and compared with light intensity, light penetration, and temperature profiles.

When ultraviolet light is absorbed by humic substances, superoxide ($O_2^{-}\cdot$), a negatively charged radical and other reactive intermediates are formed (Larson et al. 1981; Baxter and Carey 1983; Draper and Crosby 1983a,b; Cooper and Zika 1983; Petasne and Zika 1987; Zepp et al. 1987a). In situ formation of H_2O_2 results from a disproportionation of $O_2^{-}\cdot$. Algal H_2O_2 production has also been reported (Palenik et al. 1987; Zepp et al. 1987b) but the relative importance of this pathway is not known.

Because H_2O_2 is an oxidizing agent, it is not surprising that it has been shown to influence chemical (Draper and Crosby 1984; Zepp and Baughman 1978; Zika and Cooper 1987, and references therein; Zepp et al. 1987b) and biological processes in marine waters (Van Baalen 1965; Morse et al. 1977). Studies of the oxidation of iron (McMahon 1967, 1969; Collienne 1983; Miles and Brezonik 1981), manganese (Sunda et al. 1983), and chromium (van der Weijden and Reith 1982), suggest that these metals may also play a role either in the formation or decomposition of $O_2^{-}\cdot$ and H_2O_2. Moffett and Zika (1983, 1987a,b) have discussed the formation of H_2O_2 in sea water resulting from the redox cycling of copper and iron. $O_2^{-}\cdot$ is known to react with organic chemicals (Bielski et al. 1985; Rotilio 1986), may be important in the degradation of some organic pollutants (Cooper et al. 1989) and can have adverse effects on subcellular processes (Rotilio 1986). The relative stability of H_2O_2 and simple methods for its measurement enable it to be used to study $O_2^{-}\cdot$. Such information is a prerequisite for insight into metal speciation, the fate and transport of organic pollutants, as well as survival and behavior of organisms in freshwater.

Methods

Lake water samples were collected in 6-L Niskin bottles on a Rosette sampler equipped with an electronic bathothermograph. Station locations are given along with related physical and chemical measurements (Table 1). Particulate organic carbon (POC) and particulate nitrogen analyses were conducted using a Hewlett Packard CHN elemental analyzer. Samples were collected by filtering 100–400 mL lake water through

TABLE 1. Location, physical, and chemical features of the study sites. Epilimnetic concentrations of particulate material and dissolved nutrients ($\mu g \cdot L^{-1}$) at study sites.

	Ontario	Erie East	Central	Erie West	Jack's Lake
Station No.	212	23	84	357	Williams Bay
Latitude	43°23′44″	42°29′59″	41°56′01″	41°43″20″	44°41′20″
Longitude	79°24′52″	79°54′06″	81°39′49″	81°39′40″	78°02′54″
Date	10 Aug.	11 Aug.	12 Aug.	13 Aug.	6 and 8 Aug.
Depth (m)	100	65	23	10	21
PAR ext. coef. (m^{-1})	0.38	0.27	0.28	1.21	0.48
400 nm ext. coef. (m^{-1})	0.53	0.38	0.42	2.30	0.52
Chlor *a*	2.5	0.8	0.8	25	3.5
POC	609	320	445	1480	560
C:Chl	244	400	556	59	160
Part. P	6.6	3.7	3.0	26	2.1
Total P	13.0	8.1	8.0	37	6.1
Soluble reactive P	1.2	0.8	0.2	2.5	<0.2
NH_4	14	12	9	26	5
NO_3	150	130	120	25	12

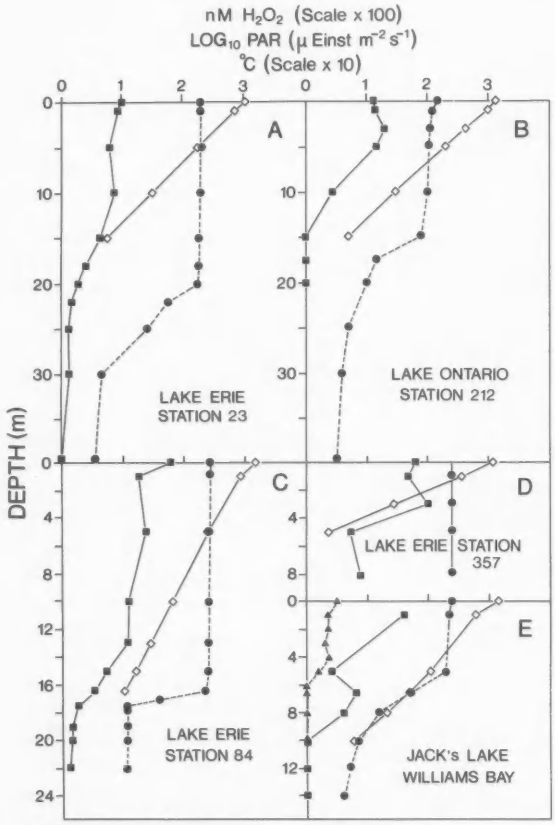

FIG. 1. Depth profiles for H_2O_2 scale × 100 = nM, (■); $\log_{10}$ photosynthetically active radiation (PAR) (◇); and temperature (scale × 10 = °C) in degrees centigrade (●) for (A) Lake Erie, station 23 in the eastern basin on 11 Aug. 87; (B) Lake Ontario, station 212 on 10 Aug. 87; (C) Lake Erie, station 84 in the central basin on 12 Aug. 87; (D) Lake Erie, station 357 in the western basin on 13 Aug. 87; (E) Jacks Lake, Ontario, collected on 6 Aug. 87 (■) and 8 Aug. 87 (▲). The depth of the water column at the five stations was 65, 100, 23, 10, and 21 m, respectively.

preignited (525°C) Whatman GF/C filters which were then dried by vacuum desiccation. Chlorophyll *a* (uncorrected for phaeopigments) was determined using the 90% acetone extraction method. Total phosphorus was measured after digestion with sulfurous acid. Particulate phosphorus was obtained by difference between results for unfiltered and filtered (0.45 μm Sartorius 11103 cellulose nitrate) samples (Environment Canada 1979).

Photosynthetically active radiation (PAR), 400–700 nm, was measured using a Li-Cor 185 Quantum Sensor. A QSM2000 quanta spectrometer (Techtum Instruments, Sweden) was used to determine spectral composition of light with depth and the extinction coefficient for 400 nm.

H_2O_2 was analyzed using the scopoletin horse radish peroxidase fluorescent decay method (Kieber and Helz 1986; Holm et al. 1987; Cooper et al. 1988). In our experiments, the limit of detection was 5 nM. A Turner Designs Model 10 fluorometer equipped for excitation and emission wavelengths of 365 and 490 nm, respectively, was used. Replicate analyses on the same sample were within ± 10% at concentrations of 50 nM.

H_2O_2 is a powerful oxidizing agent and can be lost as a result of either chemical or biological processes. Dark H_2O_2 decay was obtained by measuring the H_2O_2 concentration of samples stored in 300-mL BOD bottles at zero time and approximately every 4 h for the next 16–20 h. The samples were incubated at the temperature of the depth sampled and not stirred except prior to analyses. The bottles had been previously acid washed, rinsed several times with boiling water and finally distilled water. Apparent first order rate constants were calculated from the slope of ln H_2O_2 concentration plotted as a function of time.

Results

Vertical Gradients of H_2O_2

At the deepest part of the eastern basin of Lake Erie, station 23, H_2O_2 decreased from values near 100 nM at 0–10 m, to <20 nM at the bottom of the 20-m deep epilimnion (Fig. 1A). A slight but measurable thermal gradient, less than 0.4°C, existed between 10 and 20 m. UV radiation does not penetrate lake water as well as the longer wavelengths and here the 1% PAR light level was at 13 m. Nevertheless measurable H_2O_2 was observed even to 30 m.

In contrast to the above example, the H_2O_2 concentration in Lake Ontario (10 Aug 1500 h) declined from subsurface maxima

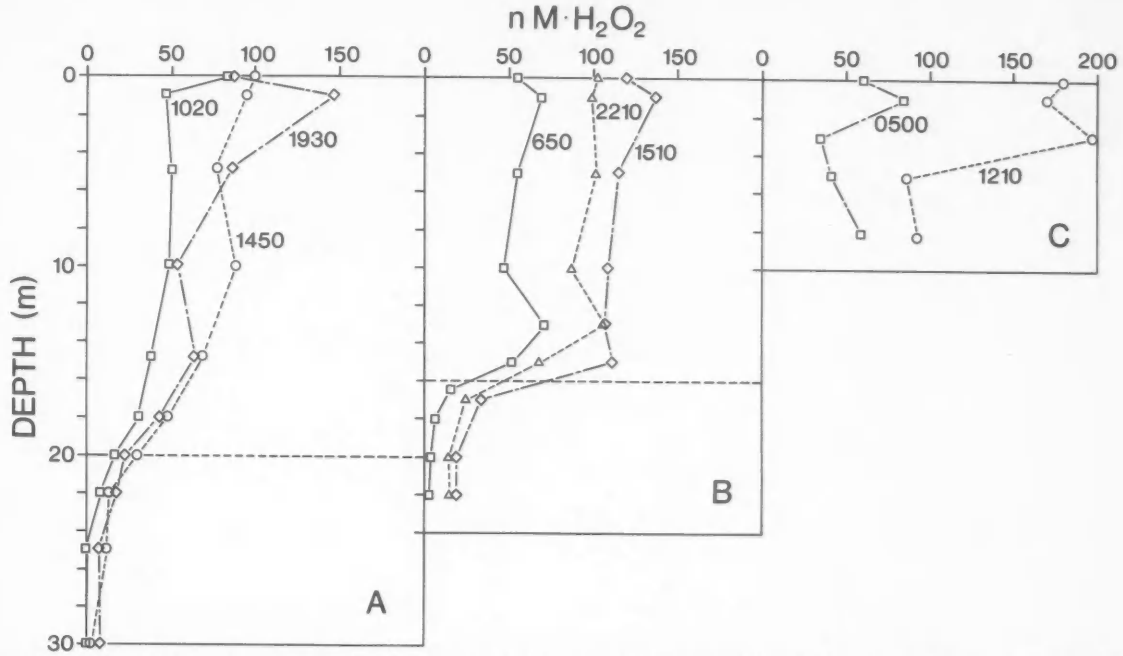

FIG. 2. Examples of H_2O_2 profiles throughout the day in Lake Erie (A) station 23, (B) station 84, and (C) station 357 on Aug. 11, 12, and 13, respectively. The bottom of the epilimnion is shown with a broken line.

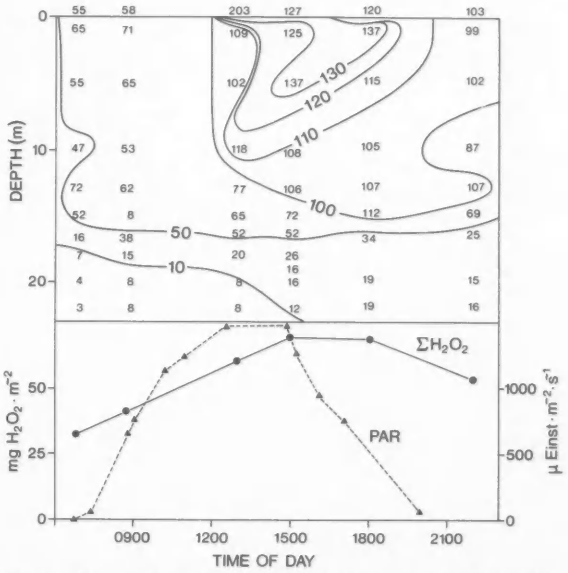

FIG. 3. Top panel; isopleths of H_2O_2 concentration (H_2O_2 in nM) throughout the water column at Lake Erie, station 84 on 12 Aug. 87. The broken line denotes the bottom of the epilimnion. Bottom panel; integrated values for H_2O_2 from data in the upper panel. Also shown is incident PAR.

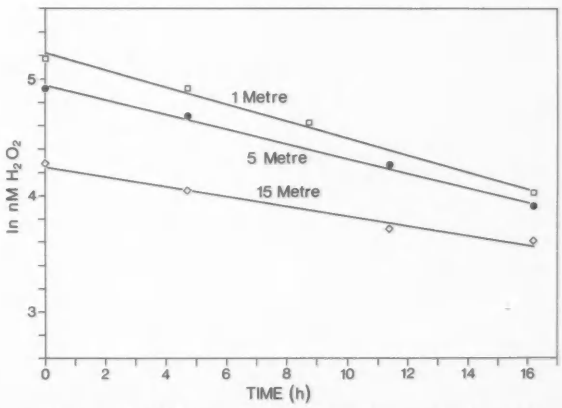

FIG. 4. Graph of ln H_2O_2 concentration in bottles as a function of time for central basin Lake Erie, station 84, water from 1 (□), 5 (●), and 15 (◇) m.

concentrations of 130 nM to undetectable at 15 m (Fig. 1B). Here the temperature change was about 1.5°C down to 15 m. The depth of 1% PAR was 11.5 m.

In the central basin of Lake Erie, station 84, a decline in H_2O_2 concentration was observed across the epilimnion even though the thermal gradient was insignificant in the upper 16 m (Fig. 1C). The 1% PAR depth was 16 m, so UV penetration to these depths would be unlikely.

In the eutrophic western basin of Lake Erie, the water column was isothermal at 24°C and the 1% PAR was only 3.7 m. The H_2O_2 concentration was fairly constant through the top 3 m but was still present at half the surface concentration at 5–8 m (Fig. 1D).

In Jacks Lake, a metalimnetic chlorophyll *a* maxima (12 $\mu g \cdot L^{-1}$) occurred at the same depth as elevated H_2O_2

TABLE 2. Apparent first order decay rate constants for H_2O_2 from stations in Lake Ontario and the central basin of Lake Erie. Also provided are the initial concentrations (H_2O_2), half lives, and the chlorophyll *a* concentration.

Depth (m)	(H_2O_2) (nM)	k (h^{-1})	$t_{1/2}$ (h)	Chlor *a* ($\mu g \cdot L^{-1}$)
Lake Ontario (station 212)				
0	112	0.047	14.7	2.7
1	114	0.046	15.1	2.8
3	130	0.047	14.7	2.5
5	116	0.040	17.3	2.2
10	44	0.032	21.6	2.9
Lake Erie (station 84)				
0	177	0.072	9.6	0.4
1	125	0.052	13.3	0.9
5	137	0.062	11.1	0.7
10	108	0.065	10.2	0.5
13	106	0.060	11.6	0.5
15	72	0.043	16.3	0.5
16.4	52	0.034	20.2	0.4

concentrations on 6 August (Fig. 2E). Epilimnetic chlorophyll *a* concentrations at this time were 0.9 $\mu g \cdot L^{-1}$ but following rain and cloudy conditions on 7 August, chlorophyll *a* increased to 3.5 $\mu g \cdot L^{-1}$ in the surface water, H_2O_2 concentrations were lower in the upper 4 m and no metalimnetic H_2O_2 was observed on 8 August. Such observations suggest that metalimnetic H_2O_2 production occurred but more rigorous experiments are required.

Diel patterns of H_2O_2 concentration

Examples of changes observed during the day (Fig. 2) for the three basins of Lake Erie illustrate that H_2O_2 not only was distributed rapidly throughout the upper mixed zone but increased in the metalimnion and even the hypolimnion (Fig. 2A,B). Whether it was produced or somehow physically transported to these depths is not known. If in situ production of superoxide, and consequently H_2O_2 depends on UV light, it would be impossible for the H_2O_2 to be produced at these depths. Despite the isothermal conditions at the western basin station (Fig. 2C), H_2O_2 increased most above 3 m but doubled at 5 and 8 m.

Values obtained from a more detailed examination of diel changes in concentration of H_2O_2 were integrated over the water column and expressed in areal units (Fig. 3). As PAR increased after sunrise, so did H_2O_2 but the latter peaked after the midday PAR maximum. At sunrise an amount of H_2O_2 equal to about half the daily maximum exists. This quantity is the result of not only what was produced the day before, but also the rate of overnight decay.

The depth for H_2O_2 production remains unknown and surface concentrations alone fail to provide the total production as H_2O_2 mixes downward in the upper mixed zone. Decay processes also reduce the observed concentration such that the observed accumulation represents the net rate not the formation rate. The areal accumulation rate for the central basin (Fig. 3, lower panel) was 4.8 $mg \cdot m^{-2} \cdot h^{-1}$ calculated from 0650 to 1500. Using profiles provided on Fig. 3, areal rates of accumulation for the eastern and western basins were 6.8 and 3.8 $mg \cdot m^{-2} \cdot h^{-1}$, respectively.

Decay of H_2O_2

To distinguish decay losses of H_2O_2 from diffusive (mixing) losses, water samples were incubated in the dark in 300-mL BOD bottles. Analyses for H_2O_2 were conducted approximately every 4 h. Examples from the central basin of Lake Erie are shown on Fig. 4 with all data summarized on Table 2. When samples were filtered through 0.45-μm membrane filters, H_2O_2 concentrations did not change over a 7-h period. Freshly collected sediment added to a water sample from 5 m to give concentrations of 8.6, 17.2, and 43 mg dry wt $\cdot L^{-1}$ did not alter the rate of H_2O_2 loss. This indicates that sediment associated reduction was not a likely cause for the H_2O_2 decay and supports the hypothesis that bacteria and/or algae are the likely agents for the decline in H_2O_2 concentration.

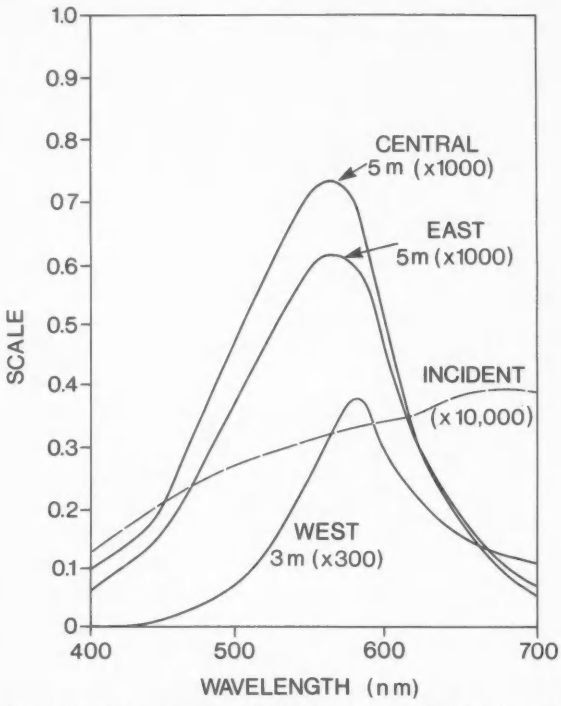

FIG. 5. Spectral composition of incident light and light at 5 m for each of the study sites in Lake Erie. Measurements were made near solar noon with a cloudless sky. The quanta ($q \cdot m^{-2} \cdot nm^{-1}$) is obtained by multiplying the scale by the factor shown in brackets.

Light quality

The spectral composition of the light (Fig. 5) changed with depth in the water column. At all three basins in Lake Erie (and Lake Ontario, data not shown), the blue (400 nm) and the red (700 nm) was absorbed to a greater extent than the green in the 560–580 nm range. Further investigations should include measurements in the near UV 300–400 nm range.

Discussion

Since areal production in H_2O_2 increased with light intensity, photochemical or photoautotrophic processes are responsible. Furthermore, the yield of H_2O_2 by photochemical production decreases with increasing wavelength (Cooper et al. 1988). If H_2O_2 formation is restricted to the depth of near UV penetration, then H_2O_2 provides a tracer to monitor physical transport of water masses. Alternatively, if other means of H_2O_2 pro-

duction exist, such observations provide clues for further experimental investigations.

Photoautotrophic H_2O_2 production may be significant deep in the water column. Zepp et al. (1987b) found production of H_2O_2 in algal cultures (40–800 $nM \cdot L^{-1} \cdot h^{-1}$) that was related to light intensity and algal abundance but different algal cultures formed H_2O_2 with different efficiencies. Palenik et al. (1987) reported that a marine alga, *Hymenomonas carterae*, produced H_2O_2 under low light conditions.

In the central basin of Lake Erie sediments are mixed with surface waters during periods of high winds (Charlton and Lean 1988). Preliminary experiments were conducted to determine if concentrations of H_2O_2 could be altered by the presence of surficial sediments. No effect was detected.

The decay of H_2O_2, observed in this study, is an apparent first order process that is effectively stopped when samples are filtered through 0.45-μm filters. Decay is therefore associated with suspended particulate materials, presumably phytoplankton and bacteria. Decay of H_2O_2 has been observed in cultures of fresh water green algae and cyanobacteria (Zepp et al. 1987b).

Patterns of superoxide and hydrogen peroxide production must be better understood for they certainly play a central role in the chemical transformations of not only metals and naturally occurring organic materials, but also of man made pollutants. Clearly, additional studies are required before a detailed understanding of the impact of H_2O_2 and related processes on lake ecosystems will evolve.

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Factors Influencing the Abundance of Blue-Green Algae in Florida Lakes[1]

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Canfield, D. E., Jr., E. Phlips, and C. M. Duarte. 1989. Factors influencing the abundance of blue-green algae in Florida lakes. Can. J. Fish. Aquat. Sci. 46: 1232–1237.

Phytoplankton samples collected from 165 Florida lakes were examined to determine relationships between blue-green algal abundance and environmental conditions. Blue-green algal biomass in the Florida samples was weakly correlated ($r = -0.34$) with water transparency and the concentration of total nitrogen (TN) ($r = 0.47$) and total phosphorus (TP) ($r = 0.33$). The relative contribution of blue-green algae to total phytoplankton biomass, however, did not decrease with TN/TP>29. Blue-green algal biomass was strongly correlated ($r = 0.90$) to total algal biomass, and blue-green algae became consistently dominant when total algal biomass exceeded 100 mg/L.

On a examiné des échantillons de phytoplancton recueillis dans 165 lacs de Floride pour déterminer les relations existant entre l'abondance des algues bleues et les conditions du milieu. On a trouvé qu'il y avait une faible corrélation de la biomasse des algues bleues présentés dans les échantillons de Floride avec la transparence de l'eau ($r = -0,34$), de même qu'avec la concentration d'azote total ($r = 0,47$) et de phosphore total ($r = 0,33$). La proportion relative des algues bleues par rapport à la biomasse totale du phytoplancton n'a cependant pas diminué lorsque le rapport AT/PT était supérieur à 29. On a établi une forte corrélation ($r = 0,90$) entre la biomasse des algues bleues et la biomasse totale des algues; on a trouvé aussi que les algues bleues constituaient à tout coup le groupe d'algues dominantes lorsque la biomasse totale des algues dépassait 100 mg/L.

Received July 5, 1988
Accepted March 9, 1989
(J9801)

Reçu le 5 juillet 1988
Accepté le 9 mars 1989

Many of the undesirable consequences of lake eutrophication are linked to the development of excessive populations of blue-green algae (e.g. Lund 1969; Reynolds and Walsby 1975; Gregor and Rast 1980). Extensive research on blue-green algae has generated several hypotheses concerning the relationship between environmental conditions and blue-green algal abundance: (1) the concentrations of monovalent cations (sodium and potassium) influence blue-green algal abundance (Provasoli 1969); (2) blue-green algae become dominant because of their ability to use bicarbonate more efficiently than other algal species, thereby enabling them to photosynthesize at lower carbon dioxide concentrations than other algal species (King 1970; Shapiro 1973, 1984; Paerl and Ustach 1982); (3) the contribution of blue-green algae to the biomass of phytoplankton is influenced by the underwater light climate (Mur et al. 1978; Smith 1985, 1986); (4) eutrophic waters are more likely to have planktonic blue-green algae than oligotrophic waters (e.g. Lund 1969; Wetzel 1975; Trimbee and Prepas 1987); and (5) the relative abundance of blue-green algae is primarily determined by ratios of nitrogen to phosphorus in the water (Schindler 1977; Smith 1983).

Many Florida lakes are mesotrophic or eutrophic due to edaphic factors (Canfield 1981; Canfield and Hoyer 1988). Blooms of blue-green algae are common in many of these lakes and some of the blooms reach enormous (310 km^2) sizes as happened in Lake Okeechobee during 1986 (Jones 1987). Consequently, there is a great deal of interest in manipulating environmental conditions (e.g. nutrient control; alterations of N/P) in order to control nuisance blue-green algal blooms. Our purpose here is to evaluate some of the more prominent hypotheses (see above) regarding the factors influencing the abundance of blue-green algae as they may apply to Florida lakes.

Methods

Phytoplankton data presented in this study were collected during a limnological survey of 165 FLorida lakes that spanned a wide range of tropic and chemical conditions (Canfield 1981, 1983; Canfield et al. 1985). Phytoplankton were sampled once in the spring (March to June) and once in the summer (July and August) of 1980. On each sampling date, surface water (0.5 m) samples were collected at each lake from three midlake locations. At larger lakes like Lake Okeechobee, samples were collected from three near-shore, open-water areas. Prior to being transported to the laboratory for analysis, water samples were placed on ice and algal samples were preserved with Lugol's solution and stored in the dark (APHA 1976).

At the laboratory, pH was measured by use of an Orion Model 601A pH meter calibrated against buffers at 4.0, 7.0, and 10.0. Total alkalinity (mg/L as $CaCO_3$) was determined by titration with 0.02 N sulfuric acid (APHA 1976). All samples were titrated to a pH of 4.5 to standardize titrations and avoid interference from silicates, phosphates, and other materials (APHA

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1976). Reported alkalinities, therefore, may be slightly greater than true alkalinities in some lakes because the equivalence point occurs at pH >4.5 in low alkalinity samples and many Florida lakes have low total alkalinities. Carbon dioxide concentrations were calculated using the equation of Saunders et al. (1962). All CO_2 values were corrected for water temperature. Specific conductance (microSiemens per centimetre at 25°C) was measured by using a Yellow Springs Instrument Company Model 31 conductivity bridge. Total phosphorus concentrations (milligrams per cubic metre) were determined by using the procedures of Murphy and Riley (1962) with a persulfate digestion (Menzel and Corwin 1965). Total nitrogen concentrations (milligrams per cubic metre) were determined by using the modified Kjeldahl technique described by Nelson and Sommers (1975).

Water samples were analyzed for color, sodium, and potassium following filtration through Gelman Type A-E glass fiber filters. Color (Pt–Co units) was determined by using the platinum–cobalt method and Nessler tubes (APHA 1976). Sodium and potassium concentrations (milligrams per litre) were determined by flame photometry (APHA 1976).

The concentration of plankton algae in each lake was estimated by measuring chlorophyll *a* concentrations. A measured volume of lake water was filtered through a Gelman Type A-E glass fiber filter. Filters were stored over desiccant and frozen until analyzed. Chlorophyll *a* concentrations (milligrams per cubic metre) were determined by the methods of Richards and Thompson (1952) and Yentsch and Menzel (1963). Chlorophyll *a* values were calculated using the equations of Parsons and Strickland (1963) without corrections for phaeophytin.

Total algal volumes in the phytoplankton samples were calculated from cell counts and size measurements. Algal samples were concentrated by centrifugation prior to microscopical examination in a Palmer cell. At least 20 random microscopic fields were examined for each sample. Additional fields were counted when cell counts were less than 100 cells. A Nikon phase-contrast microscope was used at 400×. Algae were identified to genus and cell volume estimated from cell dimensions by approximations to the nearest geometrical shape (Edler 1979). Phytoplankton biomass was estimated from total biovolume by assuming a specific density of 1.

We used sampling dates as our unit of analysis. Because the limnological variables we sampled spanned several orders of magnitude, all data except the relative biomass of blue-green algae were transformed to their natural logarithms prior to statistical analysis. The relative biomass of blue-green algae was estimated as their percent contribution to the total community biomass. This variable was arcsin transformed prior to statistical analysis.

Results and Discussion

The lakes sampled represented a wide range of trophic conditions, water chemistry, phytoplankton biomass, and community structure (Canfield 1983; Canfield et al. 1985; Canfield and Hoyer 1988). The lakes, however, tended to be eutrophic (mean chlorophyll $a = 18$ mg/m^3). Water transparency was generally low (mean Secchi disc = 1.8 m) because of high algal biomass and color (mean color = 52 Pt–Co units) concentrations (Canfield and Hodgson 1983).

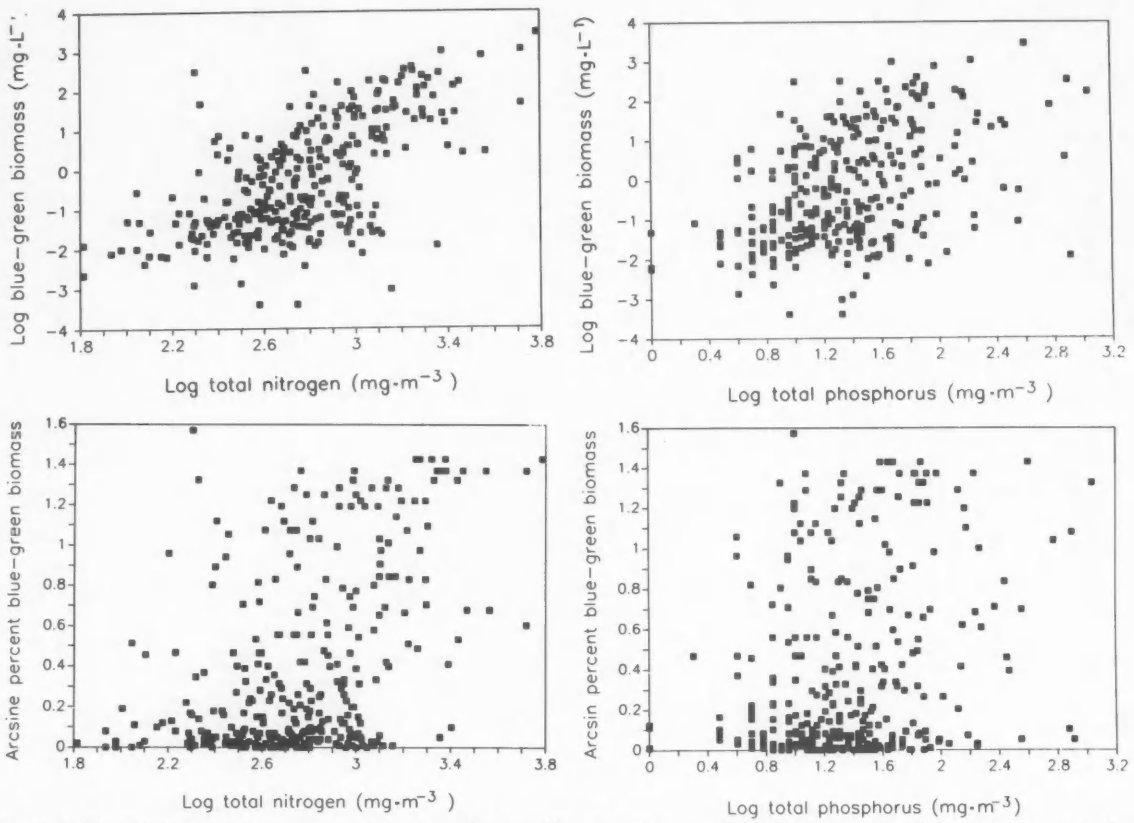
The contention that high concentrations of monovalent cations (sodium and potassium) favor blue-green algal dominance (Provasoli 1969) was not supported by our data. Neither the absolute concentration of sodium and potassium, nor their relative concentration (as the ratio of sodium + potassium to specific conductance) were significantly correlated to the relative abundance of blue-green algae.

Water transparency was weakly ($r = -0.34$; $p<0.01$) correlated to the relative biomass of blue-green algae. Although previous investigators have stressed that underwater light climate should be estimated using both Secchi disc transparency and the depth of the mixed layer, the possible effects of the depth of the mixed layer were not determined because this study used data from individual sampling dates rather than growing season averages. Most Florida lakes also have very shallow (<3 m) mean depths and are well mixed. The negative relationship between water transparency and the relative biomass of blue-green algae, however, agrees with Smith (1986), but the interpretation of this pattern is confounded by the negative relationship ($r = -0.49$; $p<0.01$) between water transparency and total phytoplankton biomass. The possible spurious nature of the correlation between water transparency and the relative biomass of blue-green algae is manifested by the lack of any significant correlation between the relative abundance of blue-green algae and Secchi disc transparency once the total biomass of phytoplankton is considered (partial r between blue-green algal biomass and Secchi transparency = 0.09, $p>0.05$; partial r between blue-green algal biomass and total phytoplankton biomass = 0.65, $p<0.01$). Despite the significant contribution of humic substances to light extinction in many Florida lakes (Canfield and Hodgson 1983), color was not significantly correlated ($r = -0.07$; $p>0.05$) to the proportion of blue-green algae in our samples.

We found a weak ($r = -0.52$; $p<0.05$) correlation between the relative abundance (biomass) of blue-green algae and the total concentration of dissolved carbon dioxide. This suggests that carbon dioxide concentrations may influence the relative biomass of blue-green algae in Florida lakes. The weakness of this relationship, however, indicates that other factors are influencing the biomass of blue-green algae in the lakes.

The concept that blue-green alge tend to dominate in lakes receiving an abundant nutrient supply (Smith 1985, 1986; Sommer et al. 1986; Trimbee and Prepas 1987) was only partially supported by our data because both total nitrogen ($r = 0.47$; $p<0.05$) and total phosphorus ($r = 0.33$; $p<0.05$) were weakly correlated to the total biomass of blue-green algae (Fig. 1). The relative biomass of blue-green algae was also weakly correlated to total nitrogen ($r = 0.45$; $p<0.05$) and total phosphorus ($r = 0.26$; $p<0.05$) concentrations (Fig. 1).

The lack of strong direct correlations between nutrient concentrations and both total and relative blue-green algal biomass (Fig. 1) may result because we used data from individual sampling dates. Smith (1983, 1986) and Trimbee and Prepas (1987) based their conclusions on growing season averages, but our results do not seem to be peculiar to Florida lakes. Although blue-green algae are often found in eutrophic or hypereutrophic lakes, blue-green algae can also contribute significantly to phytoplankton biomass in mesotrophic and oligotrophic lakes (Fig. 1; Siegfried 1985; Stockner and Antia 1986). Conversely, eutrophic lakes may be dominated by other algal groups such as diatoms and green algae (Pavoni 1963; Findenegg 1966; Wetzel 1966; Jackson 1969; Januszko 1971; Lund 1973; Reynolds 1973a, b; Reynolds and Walsby 1975; Vincent 1980). The evidence that blue-green algae can dominate the cell numbers and production of algal communities of oligotrophic lakes and seas is now rapidly expanding as new methodologies allow the


Log blue-green biomass (mg·L⁻¹)
Log total nitrogen (mg·m⁻³)
Log blue-green biomass (mg·L⁻¹)
Log total phosphorus (mg·m⁻³)
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Log total nitrogen (mg·m⁻³)
Arcsin percent blue-green biomass
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FIG. 1. Relationships between blue-green algal biomass and percent blue-green biomass with total nitrogen and total phosphorus concentrations in Florida lakes.

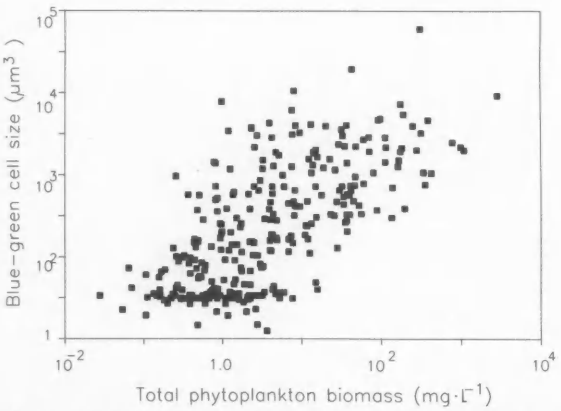

FIG. 2. Relationship between the mean size of blue-green algae and total phytoplankton biomass in Florida lakes.

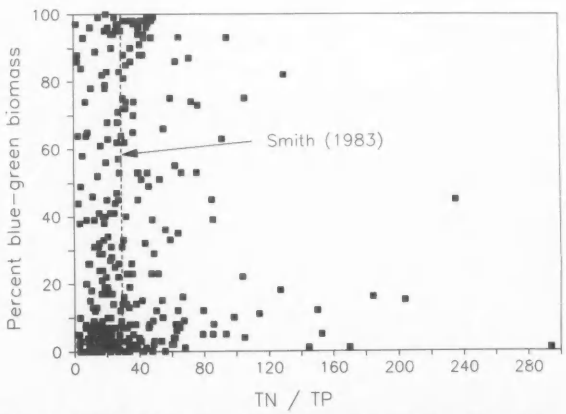

FIG. 3. Relationship between the TN/TP ratio and the relative abundance of blue-green algae in Florida lakes. The solid line shows the TN/TP ratio of 29 proposed by Smith (1983) to separate lakes with a high abundance of blue-green algae from lakes with a low abundance.

counting of small (ca. 1 μm^3) blue-green algae (Li et al. 1983; Stockner and Antia 1986; Stockner 1988). Although we did not consider picoplankton-size organisms in our lake survey, we found a relationship ($r = 0.61$; $p < 0.01$) between mean blue-green algal size and total phytoplankton biomass (Fig. 2). This suggests that the shift towards small blue-green algae as lakes become more oligotrophic is not restricted only to picoplankton-size species, but reflects instead a gradual change from large to small algae.

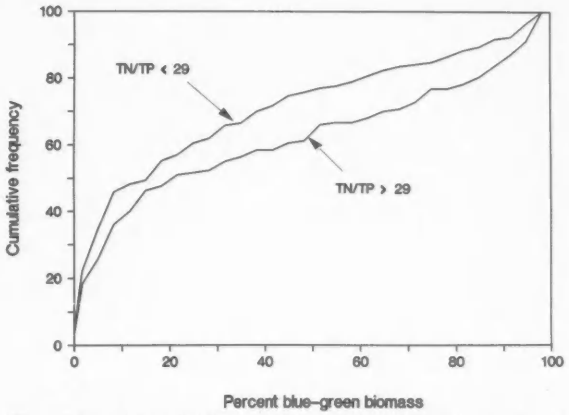

FIG. 4. Cumulative distribution of the relative (percent) biomass of blue-green algae for lakes with TN/TP ratios lower and greater than 29 by weight.

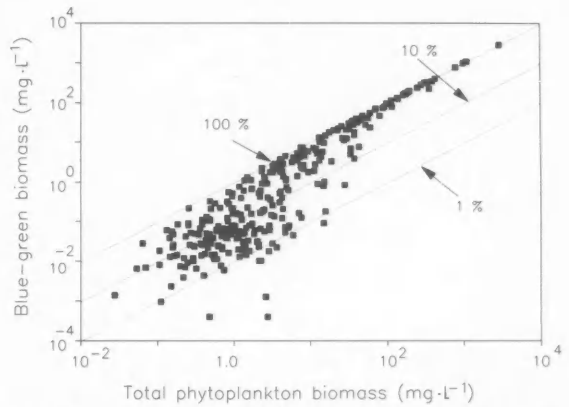

FIG. 5. Relationship between blue-green algal biomass and total phytoplankton biomass in Florida lakes. The dotted lines show the lines for which the biomass of blue-green algae equals 100, 10, and 1% of the total phytoplankton biomass.

Smith (1983) proposed that the relative contribution of blue-green algae to total phytoplankton biomass is not dependent on absolute nutrient concentrations, but rather on the ratio (by weight) of total nitrogen (TN) to total phosphorus (TP) (TN/TP). He suggested that the contribution of blue-green algae to the algal community in lakes is reduced for TN/TP greater than 29 and increases dramatically below ths ratio. Although our analyses are based on individual sampling dates rather than growing season averages, our data fail to support Smith's (1983) conclusion because many lakes with TN/TP greater than 29 have blue-green algae as the dominant phytoplankton species (Fig. 3). Further, comparison of the cumulative frequency distributions of the relative biomass of blue-green algae for lake waters with TN/TP greater than 29 and lake waters with TN/TP less than 29 indicates a weak, but significant (Kolgomorov–Smirnov test: $p<0.05$), tendency for waters with TN/TP lower than 29 to have a lower percentage of blue-green algae (Fig. 4). We, therefore, cannot support with our data the hypothesis that blue-green algae are less abundant when TN/TP exceeds 29 (also see Sommer et al. 1986: McQueen and Lean 1987).

Our results and recent evidence from a whole-lake nitrogen fertilization experiment (Lathrop 1988) suggest that the role of low TN/TP in favoring the dominance of blue-green algae has been overemphasized. The TN/TP hypothesis has been justified in part on the basis that blue-green algae are better competitors for nitrogen than other species of phytoplankton (Smith 1983) or that some blue-green algae fix nitrogen (Schindler 1977). The reason for the failure of the postulated importance of TN/TP may be that not all blue-green algae are better competitors for nitrogen (Healey 1985; Suttle and Harrison 1988) or that few eutrophic lakes exhibit a long-term absence of free inorganic nitrogen. It is more likely that short term and local depletions of nitrogen stimulate nitrogen fixation, rather than any particular average nutrient ratio. In addition, many species of bloom-forming blue-green algae (e.g. *Microcystis* spp., *Oscillatoria* spp., and *Lyngbya* spp.) do not exhibit N_2 fixation under aerobic conditions (Fay 1981; Duerr et al. 1982) and conversely nitrogen fixing species like *Anabaena flos-aquae* have high rates of growth using ammonium or nitrate as a source of nitrogen (Kerry et al. 1988; Phlips et al. 1989). The ability of some blue-green algae to compete for nitrogen or to fix nitrogen, therefore, may be a weak argument for postulating blue-green algae dominance under a TN/TP values less than 29.

Although our results suggest that blue-green algae do not necessarily tend to dominate as nutrient levels increase or in response to a specific TN/TP, there is a relationship between blue-green algal biomass and total phytoplankton biomass (milligrams per litre) in the Florida data that can be described by:

(1) ln blue-green biomass = 1.37 ln total biomass − 2.33
$R^2 = 0.81$; $n = 307$; SE intercept = 0.09; SE slope = 0.037; $p<0.05$.

This relationship suggests that blue-green algae tend to make up a higher fraction of the phytoplankton biomass as the total biomass increases (Ho: slope = 1, t-test $p<0.05$). Equation (1) is not influenced by the date of sampling or lake water temperature as would be suggested from the work of McQueen and Lean (1987) on Lake St. George (Ontario, Canada). Examination of the scatter plot for the relationship between blue-green algal biomass and total phytoplankton biomass (Fig. 5), however, reveals that there is not a uniform trend across the entire range of algal biomass. Instead, blue-green algae may represent any proportion of the total algal biomass below a total biomass of about 50 to 100 mg/L, but they become consistently dominant at biomass values greater than 100 mg/L (Fig. 5). This further indicates that although blue-green algae tend to be dominant in hypereutrophic lakes in Florida, they do not necessarily tend to be rare in oligotrophic lakes.

Blue-green algae thrive in virtually all aquatic habitats because they have, as a group, an extraordinary functional and structural heterogeneity (Carr and Whitton 1973; Fogg et al. 1973). They benefit not only from their photsynthetic ability, but from their chemotrophic and heterotrophic capabilities. This heterogeneity is a reflection of their unique place in the taxonomic mosaic and their extended evolutionary history. Our inability to adequately explain patterns in the dominance of blue-green algae through the examination of one or a few environmental variables is, therefore, not surprising.

We believe that the simple empirical models that now exist in the literature to explain blue-green algal dominance (e.g. Smith 1983, 1985, 1986, 1987; Trimbee and Prepas 1987) should be further tested to assess their general applicability. This testing is especially needed before the models are made

an integral part of the strategies for lake management (see Lathrop 1988). The strong demand for models that predict the occurrence and magnitude of algal blooms, however, must not be ignored and all efforts to develop predictive models, whatever their nature, should be encouraged.

Acknowledgments

This research was supported through a contract from the U.S. Department of Interior (Project No. 14-16-0009-79-064) and allocations to the University of Florida's Center for Aquatic Plants and Department of Fisheries and Aquaculture. We thank Mary Rutter and Lynn Hodgson for their assistance with the laboratory analyses. We also thank C.S. Reynolds and Val Smith for their constructive reviews.

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Mesoscale Aggregations of Euphausiid Sound Scattering Layers on the Continental Shelf of Vancouver Island

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Simard, Y., and D. L. Mackas. 1989. Mesoscale aggregations of euphausiid sound scattering layers on the continental shelf of Vancouver Island. Can. J. Fish. Aquat. Sci. 46: 1238–1249.

High frequency acoustics (104 kHz) and stratified (BIONESS) net tows were used to map the vertical and horizontal distribution of macrozooplankton scattering layers on and seaward of the continental shelf off Vancouver Island, in June and August of 1986. The macrozooplankton scattering layers were dominated by euphausiids, primarily *Euphausia pacifica* and *Thysanoessa spinifera*. During both surveys, dense aggregations were found in the same two general regions. The first was along the shelf break, where highest biomass was observed (averages over a few kilometres of transect reaching to about 200 mg dry weight·m^{-3} = about 40 individuals·m^{-3} and >4 g dry weight·m^{-2}). A second, broader and more diffuse aggregation occupied the deeper southeast end of the shelf (area about 200 km^2, biomass 1–2 g·m^{-2}). The scattering layers showed a consistent diel vertical migration. Daytime depth was deeper at the shelf break (~150–200 m) than on the shelf (~100–150 m) and occasionally extended to the bottom. These day-depths corresponded to the California Undercurrent waters of 6–7°C, a salinity of 33.75–34‰ and sigma-T >26.5. Distribution patterns were coincident with those of upwelled regions, high phytoplankton biomass, and high hake and herring biomass. They were also associated with the shelf break jet and the recurrent Tully eddy. There is a strong possibility of aggregation in restricted areas through the coupling of advection and vertical swimming behavior of the animals.

L'hydroacoustique a haute fréquence (104 kHz), combinée a un échantillonnage direct au moyen de filets à plancton (BIONESS), a été employée pour cartographier la répartition verticale et horizontale des couches diffusantes de macrozooplancton sur le plateau continental de l'Ile de Vancouver, ainsi qu'au large de ce dernier, en juin et en août 1986. Leur composition était dominée par des euphausides, principalement *Euphausia pacifica* et *Thysanoessa spinifera*. Des agrégations de forte densité ont été repérées dans deux mêmes régions à chaque mission. La première était confinée à la marge du plateau continental. Les plus fortes biomasses ont été observées dans cette région (moyennes sur un transect de quelques kilomètres atteignant environ 200 mg poids sec·m^{-3} = environ 40 individus·m^{-3} et >4 g poids sec·m^{-2}). La seconde agrégation, plus large et plus diffuse, était localisée dans une dépression située à l'extrémité sud-est du plateau (superficie d'environ 200 km^2, biomasse de 1–2 g poids sec·m^{-2}). Les couches diffusantes effectuaient des migrations verticales nycthémérales typiques. Leur profondeur diurne était plus grande à la marge du plateau (~150–200 m) que sur celui-ci (~100–150 m) et à l'occasion la couche diffusante s'étendait jusqu'au fond. Ces profondeurs diurnes correspondaient aux eaux du courant profond de Californie, caractérisées par des températures de 6–7°C, des salinités de 33.75–34‰ et une densité supérieure à une valeur de sigma-T de 26.5. Les agrégations coincidaient avec des régions de remontée d'eau profonde, et de fortes biomasses de phytoplancton, de hareng et de merlu. Elles étaient également associées à l'étroit courant du talus continental ainsi qu'au tourbillon récurrent de Tully. Il y a une forte possibilité que les agrégations résultent de l'action combinée de l'advection et de la nage des euphausides sur le plan vertical.

Received October 11, 1988
Accepted February 23, 1989
(J9904)

Reçu le 11 octobre 1988
Accepté le 23 février 1989

Dense and spatially localized concentrations of euphausiids are frequently observed along mid and high latitude continental margins. Concentrations of *Euphausia superba* at the Antarctic shelf break have been reported by Macaulay et al. (1984) and Shulenberger et al. (1984). Alongshore aggregations of *Thyssanoessa raschi* and *Meganyctiphanes norvegica* were observed in the Gulf of St. Lawrence (Sameoto 1976, 1983) and its estuary (Simard et al. 1986a,b). Higher concentrations of *Euphausia pacifica*, *Thysanoessa spinifera*, and *T. longipes* at shelf breaks off Oregon and British Columbia have been reported by Pearcy (1976) and Fulton and Lebrasseur (1984). Three factors contribute to the considerable importance of these populations in the transfer of energy from lower to higher levels of the pelagic food web. First, many of the important euphausiid species are omnivorous and are therefore able to utilize a wide variety of food sources (phytoplankton, smaller zooplankton, and detritus) to meet their own growth and metabolic requirements. Second, because of their relatively long lifespan (0.5–3 yr), they serve to store a portion of the productivity of lower trophic levels and smooth out some of the

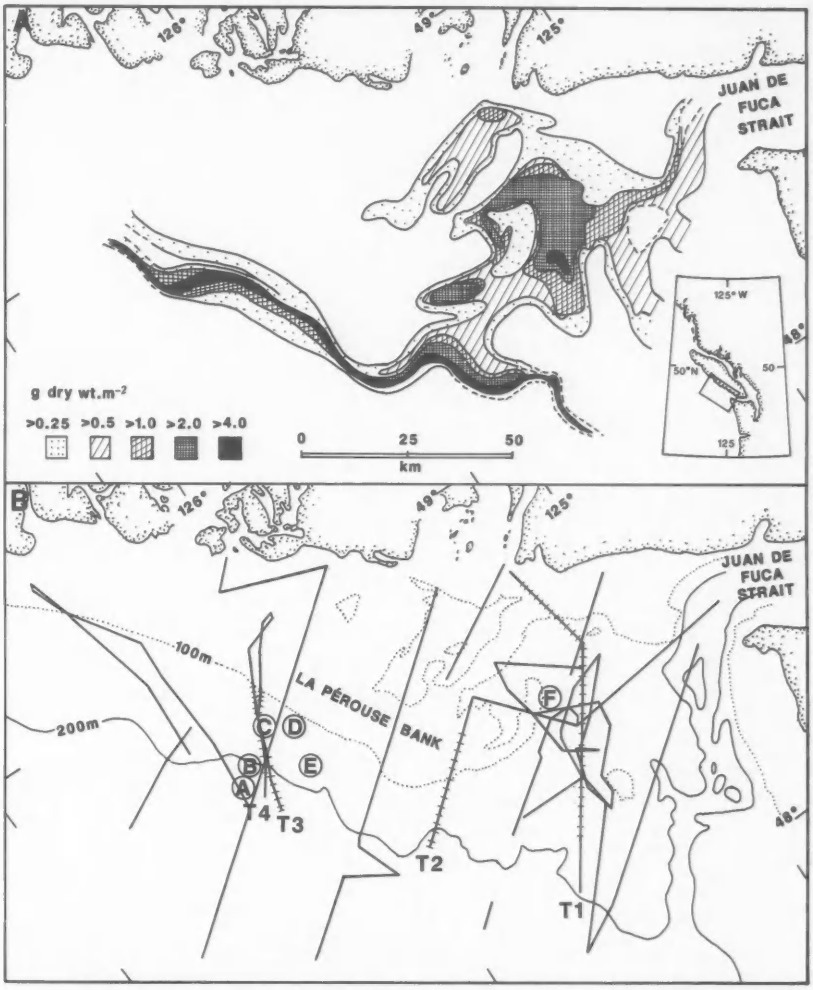

FIG. 1. Contours of the acoustical estimates of euphausiid biomass during the June cruise (A). Position of the daylight acoustic transects corresponding to A (B). The vertical distribution of biomass along hatched transects T1–T4 is presented on Fig. 3. Circled letters A–F indicate positions of Bioness tows.

intense short term and seasonal variability in their food supply. Third, because of their large individual body size (10^2–10^3 fold greater than that of a medium sized copepod) and intense spatial patchiness, they serve to concentrate a relatively large amount of potential food resource in a small volume of ocean.

The patchiness of euphausiid populations occurs over a range of spatial scales, and is almost certainly produced by a range of mechanisms. Intense small-scale aggregations (horizontal extent to about 100 m) are in general maintained by active swarming and schooling behavior; orientation and swimming speed are cued by the presence and proximity of other nearby individuals (Sameoto 1983; Hamner 1984; Nicol 1986). However, much larger scale aggregations, often several kilometres and occasionally to hundreds of kilometres in horizontal extent, also occur and can persist for many weeks (Macaulay et al. 1984; Krieger and Wing 1986; Simard et al. 1986b). These mesoscale aggregations are of major importance for feeding by whales and schools of fish, which seem to have the ability to find and utilize them (Brodie et al. 1978; Pearcy et al. 1979; Falk-Petersen and Hopkins 1981; Krieger and Wing 1986; Dolphin 1987).

Increased understanding of the mechanisms responsible for these mesoscale aggregations is fundamental to improving our ability to predict the spatio-temporal structure and stability of the pelagic food web. Scaling arguments and some field data suggest that mesoscale aggregations are more likely to result from the coupling of biological and physical transport processes rather than from purely behavioral or population growth mechanisms (Mackas et al. 1985; Simard et al. 1986b). The purpose of the present work was to study the distribution of euphausiids off Vancouver Island and the relation of this distribution to summer oceanographic conditions. This was done as a part of a large research program (the La Pérouse Project) on the oceanography and fisheries biology of the region. Specific objectives were (1) to map the distribution of euphausiids using high-frequency acoustics, (2) to identify and characterize, both spatially

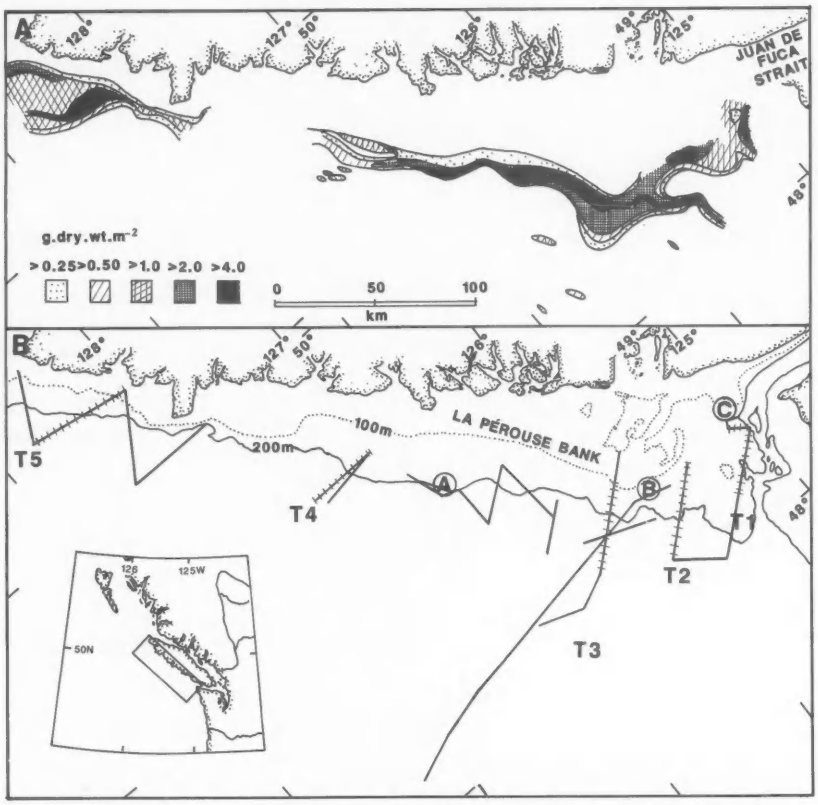

FIG. 2. Contours of the acoustical estimates of euphausiid biomass during the August cruise (A). Vertical distribution of biomass along hatched transects T1–T5 is presented on Fig. 4. Other information as in Fig. 1.

and biologically, any localized regions of high euphausiid abundance, and (3) to interpret the spatial pattern in terms of local oceanographic forcing processes.

Regional Oceanography

The continental shelf off Vancouver Island is highly productive. Nutrient rich waters are brought onto the shelf by several mechanisms including recurrent upwelling driven by the interaction of currents and bottom topography, wind-driven coastal upwelling, and the tidally-mixed outflow of Juan de Fuca Strait (Fig. 1, Mackas et al. 1980, 1982; Freeland and Denman 1982). Rich diatom blooms are frequent, often with chlorophyll *a* concentrations higher in 10 $mg \cdot m^{-3}$ (Denman et al. 1981). The phytoplankton biomass is utilized by a heterogeneous zooplankton community whose composition and population levels appear to be strongly influenced by watermass exchange processes (Mackas and Sefton 1982; Mackas et al. 1987). Euphausiids are an important component of this fauna. The core of the population appears to be centered north of Vancouver Island, on the west coast of Queen Charlotte Island and in Queen Charlotte Sound (Fulton and Lebrasseur 1984). The species dominance in winter and spring shifts from south to north from *Euphausia pacifica*, to *Thysanoessa spinifera*, and then to *T. longipes*. Euphausiids are especially abundant in deep areas of the shelf and are a primary food item for many of the commercially-important fish species (especially hake, salmon, herring, and rockfish) that inhabit the region in summer (Outram and Haegele 1972; Petersen et al. 1982; Brodeur and Pearcy 1984; Rexstad and Pikitch 1986).

Recent data on the circulation patterns off Vancouver Island have been reviewed by Freeland et al. (1984). Briefly, in winter the flow is northwest parallel to the coast. The greater speeds (30–40 $cm \cdot s^{-1}$) in the first 20 km from shore distinguish the Vancouver Island coastal current. In summer, the flow remains northwest over the inner shelf with speeds of 10–15 $cm \cdot s^{-1}$, but a southeast current (>20 $cm \cdot s^{-1}$) develops near the shelf break in response to the seasonal shift to northerly winds. The flow in the core of the underlying California Undercurrent (~200–250 m) is generally northwest at 5 to 10 $cm \cdot s^{-1}$. Dynamic instabilities of these flows and interactions with bathymetry generate meanders and mesoscales eddies on the margin of the shelf (Thompson 1984; Ikeda et al. 1984) and a recurrent cyclonic eddy on the southern end of the shelf, the Tully eddy (Fig. 8A), centered about 20 km north of Juan de Fuca canyon, from April to September (Freeland and Denman 1982; Emery et al. 1986).

Methods

Sampling was carried out from the research vessel CSS Parizeau during two cruises, 10 June–2 July and 19–28 August

TABLE 1. Composition of the macrozooplankton caught in Bioness tows in (*) and around scattering layers. Frequency (%) of occurrence, mean No., and dry weight (mg) per 100 cubic metres; mean percentage of contribution with (standard deviation) to total dry weight. Underlined No. and weight = significant higher value in scattering layer (Kruskal-Wallis test, $p<0.05$). < = <0.5; — = not measured.

	June				August			
Taxon	Frequency	Mean No.	Dry wt.	Total dry wt	Frequency	Mean No.	Dry wt	Total dry wt
Crustacea								
Euphausiacea	100*	1428*	8162*	70* (26)	100*	931*	2919*	71* (15)*
	100	354	569	26 (27)	100	127	331	45 (16)
Euphausia pacifica >12 mm	71*	791*	7196*	55* (38)*	94*	722*	1997*	45* (22)*
	53	24	182	10 (22)	75	97	280	19* (25)
<12 mm	71*	209*	82*	1* (1)*	19*	76*	81*	3* (7)*
	82	76	38	3 (3)	0			
Tessarabrachion oculatum	0				19*	3*	4*	<* (<)*
	6	<	5	1 (3)	25	1	3	< (<)
Thysanoessa spinifera >12 mm	71*	46*	409*	6* (8)*	100*	123*	821*	24* (17)*
	71	19	149	5 (6)	100	27	285	25 (15)
<12 mm	53*	383*	473*	7* (12)*	0*			
	41	233	194	6 (12)	0			
Thysanoessa inspinata	6*	<*	1*	<* (<)*	44*	9*	15*	0* (1)*
	18	1	1	< (<)	25	3	5	0 (1)
Amphipoda								
Parathemisto pacifica	59*	53*	40*	<* (1)*	81*	74*	81*	3* (4)*
	88	39	30	5 (9)	100	60	65	4 (5)
Primno abyssalis	0*				31*	2*	5*	<* (<)*
	29	2	4	1 (1)	50	2	5	< (<)
Cyphocaris challengeri	0*				19*	1*	2*	<* (<)*
	6	<		< (<)	25	1	3	< (<)
Decapoda								
Shrimp larvae	6*	<*	1*	<* (<)*	25*	1*	3*	<* (<)*
	29	1	2	< (1)	50	2	5	< (1)
Crab larvae	24*	1*	50*	1* (2)*	69*	8*	18*	1* (1)*
	41	2	3	< (<)	75	4	10	1 (1)
Copepoda	100*	—	1759*	29* (26)*	100*	—	862*	24* (14)*
	100	—	1323	68 (25)	100	—	615	47 (12)
Cirripeda larvae	24*	3*	3*	<* (<)*	0*			
	6	<	1	< (<)	0			
Mollusca								
Limacina sp.	0*				38*	8*	13*	<* (1)*
	0				75	11	15	2 (2)
Clione sp.	6*	<*	<*	<* (<)*	19*	1*	2*	<* (<)*
	18	<	1	< (<)	50	2	3	< (<)
Chaetognatha	65*	262*	—	—	69*	32*	—	—
	53	118	—	—	100	64	—	—
	n = 17 in SLs; 17 around SLs				n = 16 in SLs; 4 around SLs			

1986. Surface layer water movements were measured using drogued buoys equipped with internal Loran receivers and radio transmitters (Crawford 1988; Mackas et al. 1989). Subsurface water properties and geostrophic current estimates were obtained from a grid (see Fig. 8) of vertical profiles by a CTD/Rosette equipped with in vivo fluorometer and transmissometer (Hill et al. 1983). Chlorophyll *a* of water samples filtered on GF/C was analysed according to the standard fluorometric method (Strickland and Parsons 1972).

The zooplankton making up the scattering layers (SLs) were collected with an instrumented 0.25-m^2 mouth opening multiple net sampler (BIONESS, Sameoto et al. 1980) equipped with black nets of mesh aperture 0.23 mm. Depth of the net, in situ temperature, and cumulative volume filtered for the current net were all recorded and displayed in real time by a microcomputer deck unit. Tow speeds were approximately 3 knots and the volume of water filtered per depth stratum varied from 40 to 180 m^3. Some samples were collected in the SL and some around the SL. The zooplankton caught in these tows were analysed according to the following protocol. First, all macrozooplankton taxa were sorted, identified, counted, and weighted (dry weight per taxonomic category). The smaller juveniles euphausiids of any given species (tip of rostrum to tip of telson <12 mm) were considered separately from late juveniles and adults because of the importance of body size in determining individual acoustic target strength. The remainder of the sample (smaller zooplankton consisting primarily of copepods) was also dried and weighted. The remainder of the sample (smaller zooplankton consisting primarily of copepods) was also dried and weighted. The dry weight data were compared with corresponding acoustic data to provide an empirical conversion factor between backscatter and euphausiid biomass (see below). The analysis was done using both dry weight and number of individuals as the estimate of concentration per taxonomic category (see Table 2).

TABLE 2. Stepwise linear regressions of the acoustic estimate of zooplankton biomass (Y) against taxa (dry weight and No. per cubic metre) caught by BIONESS tows in June and August. Value of r^2 as the taxa are added to the model according to the maximum r^2 improvement algorithm, significance of the increase in r^2 (F test for H_0: increment in $r^2 = 0$; * = $p<0.05$; + = $0.05<p<0.10$; ns = $p>0.10$), and probability (T-test) that the regression coefficients are equal to zero.

	June					
	Dry wt			No.		
Taxa	r^2	F	T	r^2	F	T
Euphausiids >12 mm	0.85	*	0.00	0.85	*	0.00
Euphausiids <12 mm	0.87	*	0.01	0.86	ns	0.13
Other macrozooplankton	0.89	+	0.22	0.86	ns	0.79
Copepods[a]	0.89	ns	0.90	0.86	ns	0.81
Intercept			0.18			0.19
			$n=34$			
	August					
Euphausiids >12 mm	0.42	*	0.03	0.36	*	0.02
Other macrozooplankton	0.53	+	0.58	0.42	ns	0.11
Copepods[a]	0.56	ns	0.45	0.49	ns	0.12
Euphausiids <12 mm	0.56	ns	0.90	0.50	ns	0.53
Intercept			0.70			0.49
			$n=20$			

[a]No. of copepods = copepod dry wt/0.3 mg individual^{-1}.

Macrozooplankton vertical and horizontal distributions were sampled acoustically along the transects indicated in Figs. 1 and 2 using a system very similar to that developed by Simard et al. (1986b). A hull-mounted 104 kHz transducer (3.5° half-power beam width) was driven by a Ross echosounder. Voltage level output of the echosounder, uncorrected for absorption and spreading losses, was fed to an Apple II microcomputer via a 12 bit A/D converter. The acoustic signals were digitized in real time at a rate giving 1 m resolution over a depth range from 0 to 400 m. Horizontal averges of these depth profiles (from sequential echosounder pings) were recorded at intervals of either 30 or 120 s; the ping repetition rate was 0.54 s. Most transects were sampled at ship speed of 8–12 knots and with the longer averaging time, giving a typical horizontal resolution of 0.5–0.75 km. Calibrations against net tow catches from the SL (see below) were obtained at 3 knots and the 30 s averaging interval, giving horizontal resolution of about 50 m for these segments. The acoustic data were also recorded in echogram form on paper strip chart by the Ross echosounder. The strip chart data have much poorer resolution of echo intensity than the digitized data but much better depth and horizontal resolution. In particular, the paper records are very useful for distinguishing discrete targets (such as fish) from the more diffuse backscatter produced by macrozooplankton SLs.

The digitized acoustic data were used as in Simard et al. (1986b) to compute a relative estimate of the zooplankton biomass per cubic metre (Y) according to the standard echo integration model (Burczynski 1982):

$$I_e \propto I_o \cdot r^{-2} \cdot 10^{-2\alpha r} \cdot Y\sigma$$

For I_o and σ constant, and since $I_e \propto V^2$, we get:

$$Y \propto V^2 \cdot r^2 \cdot 10^{2\alpha r}$$

Where I_e = echo intensity; I_o = source intensity; r = range from the transducer; α = absorption coefficient of sound; Y = zooplankton biomass per cubic metre; σ = mean backscattering cross-section of a zooplankter; V = voltage level output of the echosounder corrected for system noise.

The use of a numerical correction for spreading and absorption losses instead of the echosounder time varied gain (TVG) results in a low resolution at large depths. It has, however, the advantage of better functional form and greater stability than the analog TVG, whose performance can fluctuate under intensive use during surveys. The echo integration model assumes a constant backscattering cross-section among the individual members of the SL. Clearly, such homogeneity is never encountered in the field. To partly solve this problem, multifrequency echosounding could be used (Greenlaw 1979) but was unavailable to us at the time. In practice, at a given acoustic frequency the scattering strength of a volume of water containing macrozooplankters of various sizes will be determined by the largest individuals, because the backscattered echo energy from a zooplankter increases rapidly with its body size. According to the fluid sphere model of sound scattering, it is a function of the sixth power of the radius of the organism, when the acoustic wavelength is much larger than its circumference.

In order to transform the acoustic biomass per cubic metre (Y) to mass units, linear regressions on the catch from net tows were computed. The scatter of such regression is highly dependent on the accuracy of the spatial alignment of the acoustic and net sampling (Pieper 1979; Simard et al. 1986b). Both net and echosounder need to be sampling exactly the same locations and depth strata, because the abundance of euphausiids can vary considerably over small distances. The small-scale patchiness of the SL was taken into account by increasing the spatial resolution of the digitized acoustic data during net tows (see above) and by following as accurately as possible the path of the net in the stratum. No attempt was made to include a correction for the probable avoidance of the net by a fraction (unknown) of the euphausiids (see Wiebe et al. 1982; Everson and Bone 1986).

The acoustic estimates of biomass were integrated vertically over the depth range of the macrozooplankton SL to provide estimates of biomass per square metre along the transects. It was generally possible for an experienced observer to distinguish the plankton SL from the fish aggregations by inspection of the paper echogram (see Figs. 5 and 6). In some areas, it was necessary to reduce the ship speed to get better resolution of individual targets. When significant concentrations of fish were found imbedded in the macrozooplankton SL, this part of the SL was excluded from the integration. Only daytime transects were used for quantitative mapping for three reasons: first, because depth separation of the euphausiids from other scatterers was much better in daylight; second, because nighttime acoustic target strength of euphausiids is likely to be reduced and variable due to changes in their body orientation during active vertical migration (Sameoto 1980); and third, scattering strength in the upper few metres is obscured by surface "noise." Interpolation of biomass contours between transects was done by eye based on an observed consistent (both day and night) association of population maxima with particular bathymetric contours.

Results and Discussion

Composition of the Scattering Layers

The SL biomass was dominated by the two euphausiids *Euphausia pacifica* and *Thysanoessa spinifera*, which averaged

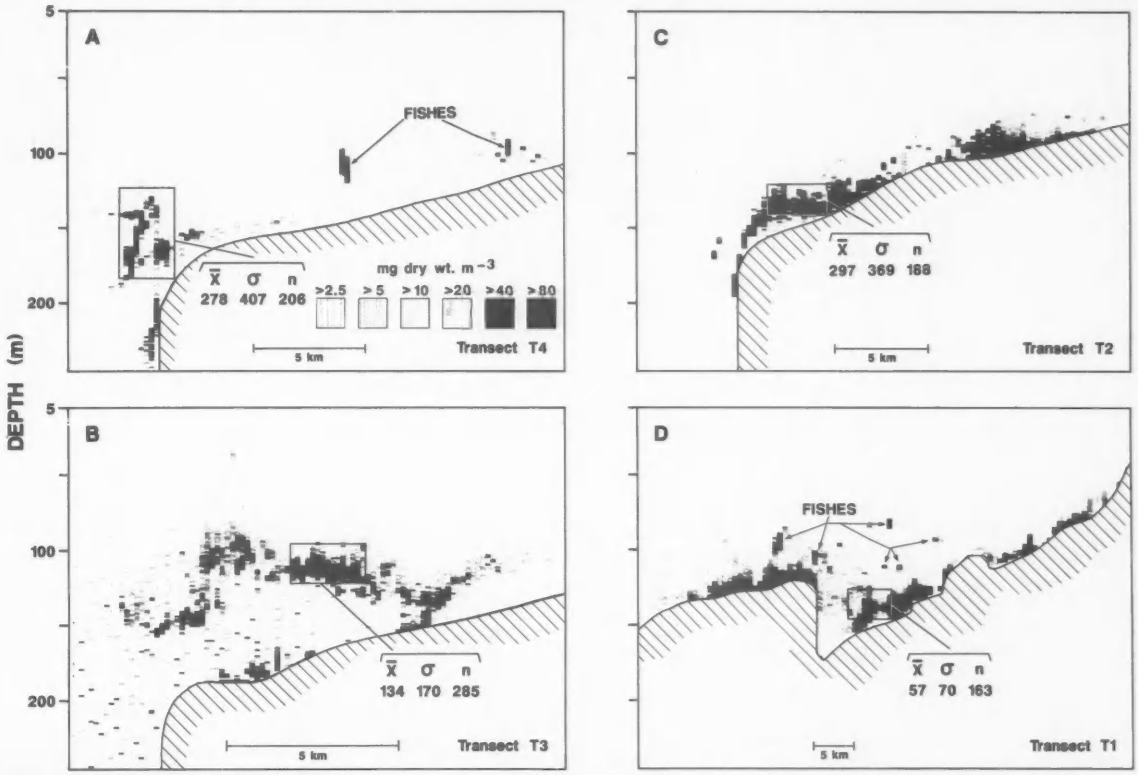

FIG. 3. Vertical distribution of euphausiid biomass in June along the transects T1–T4 (Fig. 1B). Transect T4, 12 June ~14:45–16:00 PDT (A). Transect T3, 13 June, ~21:00 back to 20:00 PDT (B). Transect T2, 2 July, ~11:45–13:15 PDT (C). Transect T1, 19 June, 20:15 back to 14:30 PDT (D). The estimated mean biomass in the SL (x, milligrams dry wt per cubic metre) with standard deviation (σ) is given for the indicated windows. Fish schools are pointed by arrows. Sunset = 21:00 PDT; sunrise = 05:00 PDT.

70% of the total dry wt of the catches (Table 1). The remainder was essentially due to copepods, mainly: *Calanus marshallae, Neocalanus cristatus, Euchaeta elongata*, and *Pseudocalanus* spp., which contributed a mean of 29 and 24% of SL dry weight in June and August samples, respectively. Chaetognaths and the hyperiid amphipod *Parathemisto pacifica* were relatively abundant but their mean contribution to SL biomass was lower than 3%. Most taxa were present both within and around SLs, but the euphausiids, especially large individuals, were the only taxa significantly more abundant in SLs. In June, the overall length-frequency distribution of the euphausiids was bimodal, with peaks at 7–9 mm and 18–20 mm. In August, a third peak around 15 mm was present in some samples. Most samples contained euphausiids belonging to more than one mode. The sex ratio of *E. pacifica* was slightly in favor of males in June samples (0.63 male, $\sigma = 0.13$) and equal to 0.50 ($\sigma = 0.12$) in August. *Thysanoessa spinifera* was caught in sufficient quantities to determine the sex ratio only in August. Its sex ratio was then highly biased towards the females (0.11 male, $\sigma = 0.02$).

Comparison of Acoustic and Net Tow Estimates of Scattering Layer Density

The largest proportion of the variance of echo intensity was explained by the large euphausiids (Table 2). The addition in the regressions of the small euphausiids, the other macrozooplankton taxa, and the copepods slightly increased the variance explained, but their regression coefficients were not significantly different from zero except for the dry weight of the small euphausiids in June. The higher percentage of variance explained in June compared with August probably results from the larger number of samples together with the sampling of denser SLs (see Table 1) and not from higher performance of the equipment.

These results again indicate the strong dominance in the SLs by the large euphausiids. This was expected because they were the most abundant taxa (Table 1) and also they had the largest body size, and hence the largest echo as noted above. At an acoustic frequency of 100 kHz the target strength of a 7-mm euphausiid is two to three orders of magnitude smaller than that of a 22-mm euphausiid (see Greenlaw 1979); the target strength of 3-mm copepods is much smaller still and their contribution to SL echos at such frequencies is negligible (see Sameoto 1983).

To estimate euphausiid biomass from the acoustic data, simple linear regressions against the dry weight of the large euphausiids only were computed, and the slopes, when forced through the origin, were used as conversion factors. Given the maximum scatter of the regression points, the error on the biomass estimates could be up to a factor of two to three.

Vertical Distribution

During both cruises, the SLs showed typical diel vertical migration, reaching the surface waters at dusk and descending

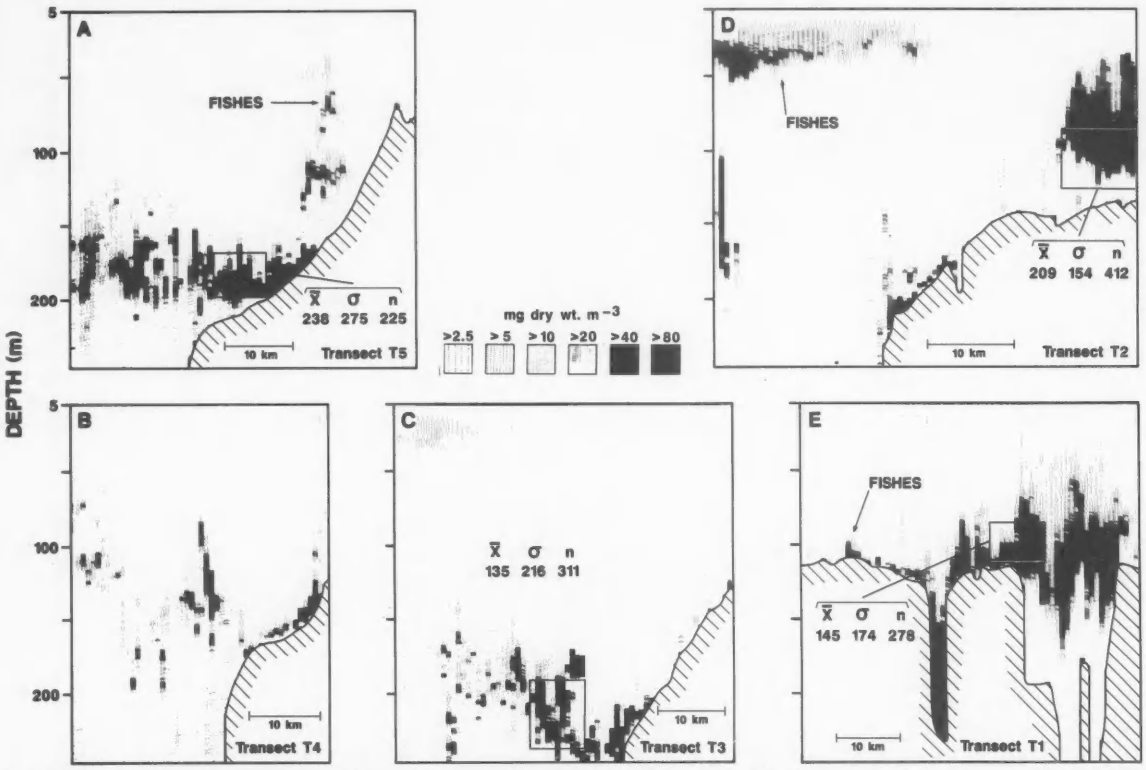

FIG. 4. Vertical distribution of euphausiid biomass in August along the transects T1–T5 (Fig. 3B). Transect 5, 19 August, ~08:00–10:15 PDT (A). Transect T4, 20 August, ~10:00–11:45 PDT (B). Transect T3, 23 August, 12:00–17:00 PDT (C). Transect T2, 28 August, 11:15 back to 06:30 PDT (D). Transect T1, 28 August, 15:40–19:30 PDT (E). Sunset = 20:00 PDT; sunrise = 06:00 PDT. Other information as in Fig. 3.

to their day-depth shortly before dawn. During daytime, the SLs were generally found at depths greater than 100 m, except for a few patches found right on the bottom in shallow areas (Figs. 3C–D, 5C). At the shelf break the day-depth was located around 150–200 m (Figs. 3A, 4A–C). It was generally about 50m shallower over the shelf (Figs. 3D, 4E). This daytime vertical distribution showed, however, a lot of small and mesoscale variability (Figs. 2B, 4C, 5A–B, 6A), some of which was clearly related to vertical displacement of subsurface strata by high frequency internal waves (e.g. Fig. 6A).

The day-depth of the SLs at both the shelf break and over the shelf seemed to correspond to the same water mass (temperature of 6–7°C, salinity of 33.75–34.00‰ and density higher than sigma-T = 26.5; see Fig. 7). These water properties are characteristic of California Undercurrent water, which is often transported onto the shelf by deep upwelling (Mackas et al. 1987). Such upwelling conditions were prevailing during the survey (Figs. 7 and 8). Even though the SLs were found in this layer during the day, their day-depth cannot be explained solely by the physical characteristics of the water masses. In fact, the vertical distribution of the euphausiids did not track the elevation of the isopycnals, isotherms, or isohalines at the shelf edge for any transect (e.g. Figs. 4C and 7C).

The euphausiids' day-depth must have been determined by some other boundary, which might be the underwater daylight. If the euphausiids were following an isolume (Kampa 1975), light attenuation by a rich bloom of phytoplankton over the shelf would have considerably raised their day-depth there. For example, an increase of the attenuation coefficient from 0.15 to 0.35 m^{-1} in a 30-m thick bloom of phytoplankton would raise the isolumes by 40 m beneath the bloom. Such conditions were encountered during the surveys. Similar arguments were used to explain localized shoaling of SLs and zooplankton in environments presenting variable penetration of downwelling light (Isaacs et al. 1974; Boyd et al. 1986; Simard et al. 1986a). Isaacs et al. (1974) have suggested that the combined effect of this behaviour of the SLs with the vertical shear in the water column might significantly help the vertically migrating organisms in finding food and maintain them in rich areas.

Because phytoplankton blooms are patchy, it is more likely that a SL following an isolume under bloom conditions, would exhibit a highly variable day-depth, with sharp changes at the edges of the phytoplankton patch. The sharp drop (50 m) in the depth of an ascending SL at sunset at the edge of the shelf shown in Fig. 3B (note: time increases from right to left) could be explained by such a mechanism. The variability of the day-depth of the euphausiid SLs under phytoplankton blooms would be an integration of the solar irradiance variability, the phytoplankton (and seston) patchiness and the local complexity of euphausiid composition, because the light levels followed vary among developmental stages and species. This could easily explain the high variability observed in the depth of the SLs during the survey, except the SLs lying on the bottom in shallow areas (Fig. 3C,D). The easiest explanation for these is that the

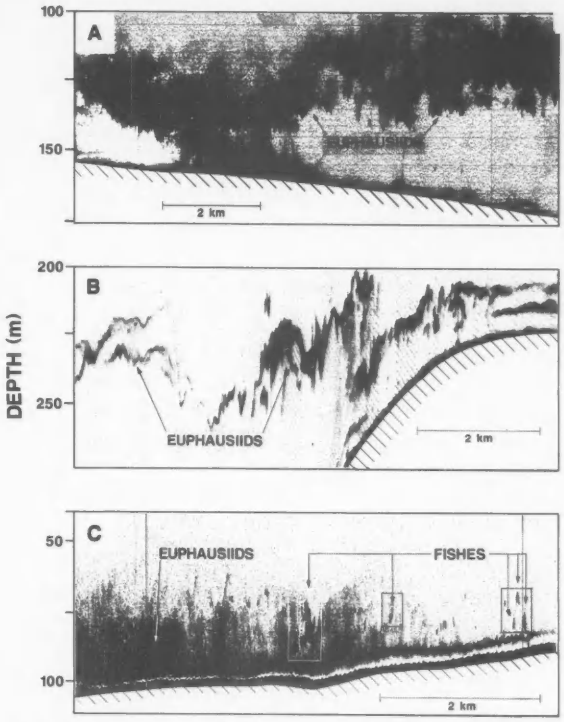

FIG. 5. Echograms of the euphausiid SLs. Dense SL of euphausiids over the shelf in June (T3) during the ascent (A). SL at the shelf break in August (B). SL in shallow depths on the shelf in June (T2) (C). Raw voltage not corrected for spreading and absorption losses. Fish schools are pointed by arrows.

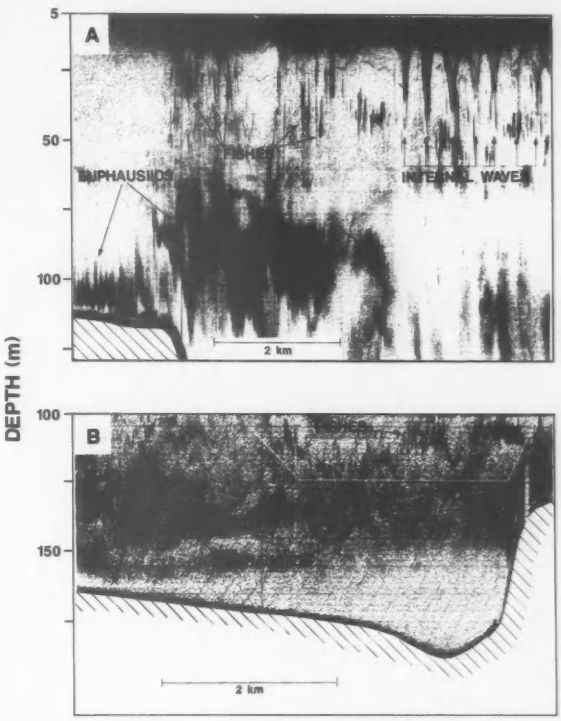

FIG. 6. Echograms of the euphausiid SLs. Dense SL around a bank at the head of the Juan de Fuca Strait in August (A). SL of euphausiid in the basin between La Pérouse Bank and the Juan de Fuca Strait in June (B). Other information as in Fig. 5.

euphausiids were trapped there by the combination of the circulation and their vertical migrations (see below).

Examples of the fine structure of the SLs are shown in Figs. 5 and 6. In general, the SLs were more patchy in August than in June, both in the horizontal (Figs. 1 and 2) and in the vertical (Figs. 3 and 4). Estimations of the biomass per cubic metre in the SLs are given for some selected windows in Figs. 3 and 4. (The statistics correspond to the portion of the window occupied by the SLs; the SLs are defined as the sum of all estimates above 5 mg dry wt·m^{-3}, and the sample unit is 1 bin of 1-m high by 120 or 30 s long.) The mean biomass over a few kilometres was often higher than 200 mg·m^{-3}. Given a mean dry weight per large euphausiid of ~5 mg (Table 1), these averages correspond to more than 40 individuals·m^{-3}. The estimates of biomass per square metre and cubic metre are in the range of values reported in several surveys (Sameoto 1976, 1980, 1983; Pieper 1979; Simard et al. 1986a,b; Falk-Petersen and Kristensen 1985), but much lower than the high concentrations encountered in some studies (Hampton 1983; Macaulay et al. 1984; Krieger and Wing 1986; Greene et al. 1988). The estimates reported here represent only one fraction of the euphausiid population, the larger individuals, which seemed to have predominantly contributed to the recorded echo. Also, an unknown proportion of euphausiids probably avoided the net and were not considered in the estimation.

Echos of fish schools were often found above shallow SLs, (Figs. 5C and 6). Off the shelf break, a layer of fishes was often present in the upper 50 m of the water column, sometimes with high intensity (e.g. Fig. 4D).

Horizontal Distribution

During both the June and the August cruises, the euphausiid SLs were concentrated (1) at the shelf break and (2) in the ~150-m deep basins located between La Pérouse Bank and Juan de Fuca Strait (Figs. 1A and 2A). The shelf break aggregation appeared to be continuous in the alongshore direction (present in 9 of 11 and 13 of 13 shelf-break crossings in June and August, respectively) over the entire ~350 km long area. It varied in width from 2–3 to ~30 km. It was sometimes confined to the margin of the shelf (Fig. 3A), but in other locations extended onto the shelf or offshore (Fig. 3B–C, 4A–C). The highest biomass per square metre was almost always within 5 km from the 200-m contour, often in a narrow patch, 2–3 km wide, where it was higher than 4 g dry wt·m^{-2}.

The basin aggregation (Fig. 1A) was more extensive and less intense than the shelf break aggregation. It covered most of the depths greater than 100 m southeast of La Pérouse Bank, including Juan de Fuca canyon (Figs. 3D, 4D–E). Although this area was poorly sampled during daylight in August (Fig. 2B), night transects in the area confirmed that the aggregation was still present in late summer. The biomass per square metre was higher in the basin south of the margin of the bank (~2 g dry wt·m^{-2}) than in the north basin (~0.5 g dry wt·m^{-2}), delimited by the 100-m contour (Fig. 1A–B). High concentrations were also found in Juan de Fuca canyon, especially in August (Figs. 2A,4E).

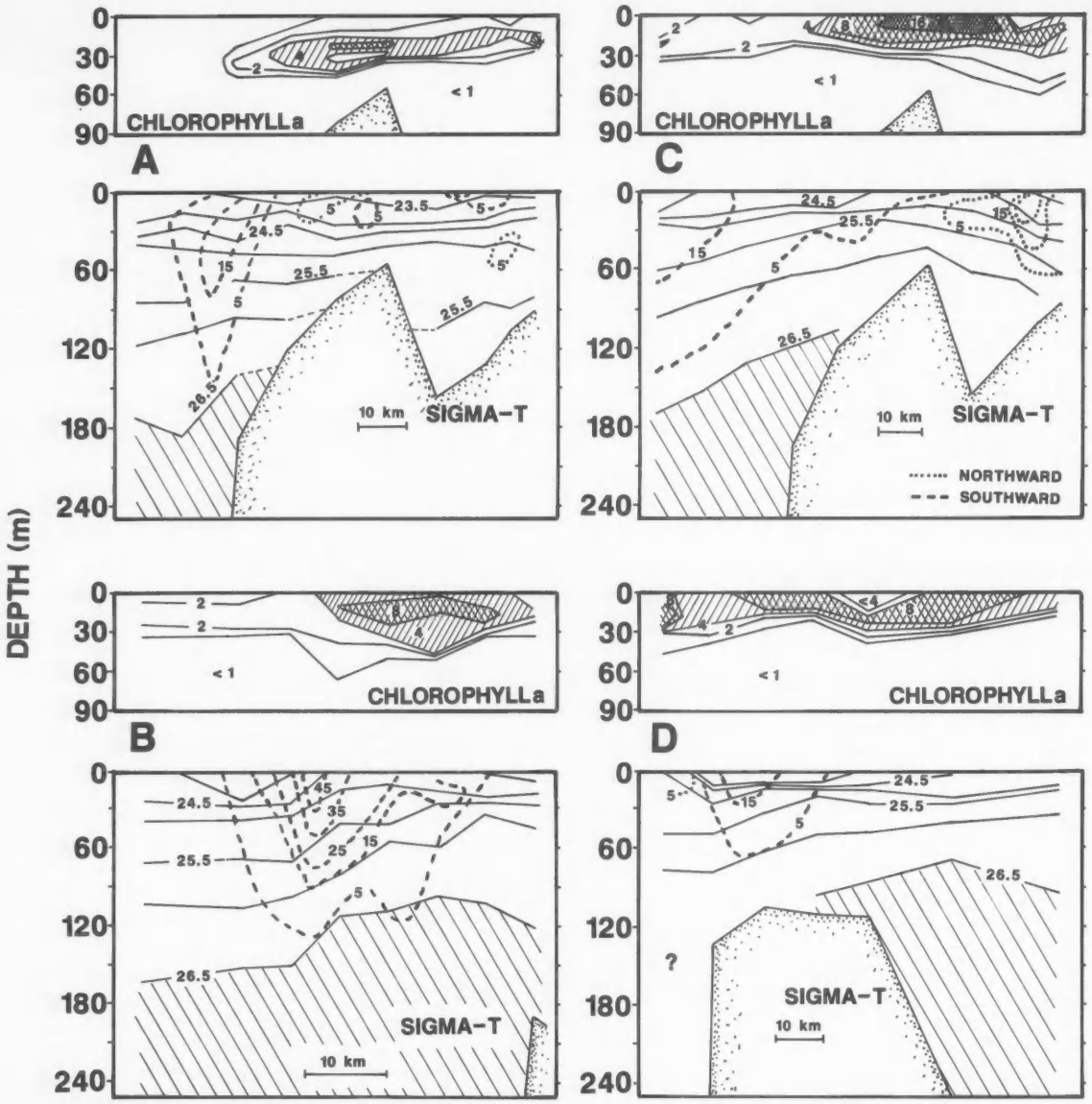

FIG. 7. Vertical distribution of chlorophyll *a* (milligrams per cubic metre), density (sigma-T) and the estimated geostrophic flow (centimetres per second) across the section, along the transects (A–D) pointed in Fig. 8.

Oceanographic Conditions

California Undercurrent water exhibited a significant intrusion over the shelf, which was more pronounced during the August cruise (Figs. 7 and 8). The upwelling was more intense at the head of Juan de Fuca Strait, in the Tully eddy region, and in the northern half of the shelf (Fig. 8). A rich phytoplankton bloom had developed in upwelling plumes, located off the shelf break in the northern half of the study area (Fig. 7B) and over the outer shelf in the southern half (Fig. 7A,C,D). The contours of the geostrophic shear across the transects (Fig. 7) illustrate typical summer upper layer flow. The two main currents are: (1) the northward-flowing Vancouver Island coastal current on the inner shelf, fed by the outflow of the Juan de Fuca Strait (e.g. Fig. 7C) and (2) the outer shelf flow which is moving southeast along the shelf break (e.g. Fig. 7A–B).

Possible Interactions between Euphausiid Distribution and Circulation Pattern

The euphausiid population was highly localized. In both June and August surveys, more than 50% of the biomass was found in less than 5% of the surveyed area (Figs. 1 and 2) even though most of it should be habitable. The lifespan of the animals (1–2 yr) is long relative to the residence time of water in the aggre-

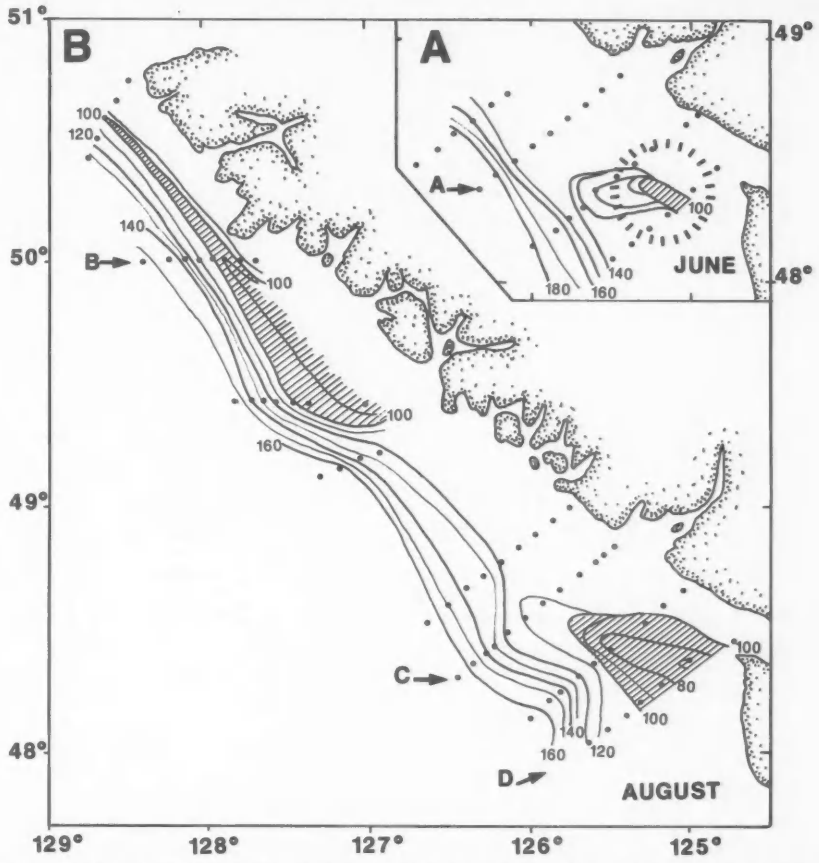

FIG. 8. Depth (metres) of the isopycnal sigma-T = 26.5 during the June (A) and the August (B) cruises. The vertical structure along transects pointed by arrows A–D are shown in Fig. 7. The radiating circle in A shows the approximate location of the Tully eddy.

gation areas (<1 wk, Freeland et al. 1984, Freeland 1988), and the horizontal currents are rapid compared with the mean swimming speed of the animals (~5 $cm \cdot s^{-1}$, Hardy and Bainbridge 1954). These factors favor a more dispersed distribution of the population and make it unlikely that the observed concentrations could be maintained by either local secondary production or by directed horizontal migration. What causes and maintains the high abundance of euphausiids in such restricted areas?

Along-shore inputs from the north help to maintain high abundance along the outer shelf. Fulton and Lebrasseur (1984) found very dense winter and spring concentrations of euphausiids along the shelf break from the north end of Vancouver Island to the southern Queen Charlotte Islands. We believe this is an important summer source pool for the euphausiid population off southern Vancouver Island. The summer shelf-break current provides an effective delivery mechanism. Strong southeast flow (>10 km per day) extends from the surface to 50–100 m depth (Fig. 7), while beneath this depth it is weak and variable in direction. The cross-shore position of the maximum current closely matches that of the peak scattering layer intensity; transport by the shelf-break current is therefore rapid whenever the euphausiids are in the upper layer. This is variable with time of day and euphausiid developmental stage. Stratified samples collected within the shelf-break current had high densities of larval and juvenile euphausiids (typical concentrations $0.3–1 \times 10^3 \cdot m^{-3}$) in the upper 15 m both day and night. Adults and later juvenile stages migrated upward into the strong current for about 8–9 h each night, but probably experienced relatively little net along-shore movement while at their daytime depth. In winter, advection from the south probably imports euphausiids from Washington and Oregon coasts.

This along-shore transport mechanism predicts a band of high euphausiid abundance extending south along the shelf break; however it does not explain the narrowness of that band nor the broader and less intense concentration maximum found on the continential shelf in the vicinity of the Tully eddy. We suggest that these features of the distribution are at least in part caused by coupling of the cross-shore circulation with euphausiid migration behavior. Throughout the region, the SLs showed typical diel vertical migration, and an underwater light level barrier (see Kampa 1975; Forward et al. 1984) seemed to control their daytime depth. Trapping of euphausiids by oblique upward advection toward an isolume barrier has been invoked to explain the aggregation of euphausiids in the St. Lawrence estuary (Simard et al. 1986b). The flow supplying the upwelling at the shelf break and in the Tully eddy (Figs. 7 and 8)

might act similarly. The euphausiids would be imported to the aggregation region by the horizontal component of flow, and concentrate there below the isolume barrier, either by sinking or by intermittent downward swimming.

The probable immediate source of the aggregation in the Tully eddy region is the Juan de Fuca canyon, where the deep upstream estuarine flow might import euphausiids from offshore. It is not clear whether this component of the population is resident or experiences large advective inputs and losses. The cross-shore advection rates at the shelf break are on average small compared with the alongshore advection. The midwater onshore flow needed to compensate wind-driven offshore transport in the surface Ekman layer is <1 cm·s^{-1} (Denman et al. 1981; Freeland and Denman 1982). Where the southeastward along-shore flow extends to the bottom, onshore flow in the bottom Ekman layer will be slightly more rapid (about 1–3 cm·s^{-1}). More work needs to be done on the possible importance of spatially and temporally intermittent cross-shore water movements associated with instability of the along-shore circulation and with irregularities in the bottom topography.

Importance for Trophic Transfers within the Pelgaic Food Web

The spatial distribution of euphausiids enhances their importance and effectiveness in transferring food energy to higher trophic levels. The aggregations are at sites with recurrent summer upwelling and dense phytoplankton blooms; the euphausiids have high probability of an adequate food supply by being located there. The spatial association also extends up the food web to their vertebrate predators. Euphausiids are locally important in the diet of several commercial fish species; hake, herring, salmon, and rockfish (Outram and Haegele 1972; Petersen et al. 1982; Brodeur and Pearcy 1984; Rexstad and Pikitch 1986; Thompson and Ware 1989). Both salmon trolling activity and rockfish abundance are concentrated along the 100 and 200-m isobaths (G. A. Borstad, Borstad Ass. Ltd, 9865 West Saanich Road, Sidney, B.C., and B. Leaman, Pacific Biological Station, Nanaimo, B.C., pers. comm.). Acoustic surveys (Taylor and Kieser 1982 and more recent unpubl. data) show that summer hake distributions are almost exactly coincident with the regions we have identified as zones of high euphausiid biomass, while herring are immediately adjacent to the euphausiid aggregations along the outer shelf and at the margin of banks. Habitat and prey partitioning between herring and hake in the region is presently the object of a more detailed study (Dan Ware, Pacific Biological Station, Nanaimo, B.C., unpubl. data). Recent work in other regions (Pearcy et al. 1979; Genin et al. 1988) has suggested that euphausiids can be advected over shallow banks during their nightly migration into the surface layer, and thereby become vulnerable to predation by pelagic and demersal fish stocks resident on the banks because they are unable to migrate back down into "safe" light levels the following morning. Examples of proximity of euphausiids and their predators are shown in Fig. 5C and 6A, where fish schools are found at the margin of a shallow bottom SL (Fig. 5C) and over a dense SL at the edge of a bank under large-amplitude high-frequency internal waves (Fig. 6A).

Acknowledgments

We thank M. Galbraith for laboratory identification and biomass analyses; R. Brown, K. Burns, and H. Sefton for shipboard help; J. Wallace, D. Farmer, and J. Thompson for loan of equipment; K. Denman and R. Forbes for access to phytoplankton data; K. Denman, H. Freeland, R. Thompson, and D. Ware for comments and discussions.

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Foraging Mode Switches in the Golden Shiner (*Notemigonus crysoleucas*)[1]

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Golden shiners (*Notemigonus crysoleucas*) use two distinct foraging modes when feeding on zooplankton. Shiners particulate-feed on large cladocerans, visually locating and attacking individual prey items. Shiners also pump filter-feed on high densities of small zooplankton, using no apparent visual cues to detect prey. Particulate-feeding and filter-feeding functional response curves were determined from laboratory experiments. Particulate-feeding rates on *Daphnia pulex* increased with shiner size, but filter-feeding rates on *Bosmina* decreased with shiner size. Experiments with both prey types present simultaneously revealed that shiners can switch between foraging modes on a short time scale which increases their total foraging return by filter-feeding on *Bosmina* in between *Daphnia* encounters. This behavioral flexibility has consequences for both fish and zooplankton community structure in winterkill lakes. The ability to switch feeding modes on a short time scale allows shiners to exist in lakes that are dominated by small zooplankton yet still provides a mechanism to effectively remove large cladocerans when they are encountered.

Le méné *Notemigonus crysoleucas* se nourrit de zooplancton selon deux modes différents. Il se nourrit à la pièce de grands cladocères qu'il repère visuellement et attaque. Il peut également filtrer le petit zooplancton qui est présent à forte densité dans l'eau, sans apparemment utiliser d'indice visuel pour le repérer. Les courbes de réponse alimentaire ont été déterminées en laboratoire. L'alimentation à la pièce (sur *Daphnia pulex*) augmentait avec la taille du *Notemigonus*, tandis que l'alimentation par filtrage (sur *Bosmia*) diminuait avec la taille du *Notemigonus*. En présence des deux proies, les ménés peuvent passer rapidement d'un mode d'alimentation à l'autre, ce qui augmente le rendement de capture de *Bosmina* entre les contacts avec *Daphnia*. Cette souplesse influe sur la structure des communautés ichtyenne et planctonique sujettes à la mortalité hivernale : elle permet aux ménés de survivre dans des lacs dominés par le petit zooplancton et de capturer efficacement les grands cladocères qui se présentent.

Received February 3, 1988
Accepted March 9, 1989
(J9574)

Reçu le 3 février 1988
Accepté le 9 mars 1989

Predation by planktivorous fishes is widely recognized to have important consequences for zooplankton community structure (Zaret 1980; Post and McQueen 1987; Lazzaro 1987). Historically, attention has focused on the size-selective effects of visually-feeding fishes that locate and attack prey items individually (e.g. Brooks and Dodson 1965), but studies have shown that the effects of nonvisual, filter-feeding fishes on plankton communities may be just as great yet qualitatively different from those of particulate-feeders (Lazzaro 1987; Drenner et al. 1982, 1986; Bohl 1982; Starostka and Applegate 1970).

A number of fish species are known to both particulate- and filter-feed (see review by Crowder 1985). Some species are particulate-feeders early in their ontogeny but then become exclusive filter-feeders after growing beyond a certain size (e.g. Michaletz et al. 1982 for paddlefish; Drenner et al. 1982b for gizzard shad). Still other species are known to use both foraging modes and facultatively switch between particulate-feeding and filter-feeding (e.g. Batty et al. (1986) for herring; Crowder and Binkowski (1983) for alewife and bloater; Janssen (1976a) for alewife; Holanov and Tash (1978) for threadfin shad; Leong and O'Connell (1969) for anchovy). Little is known about the cues that elicit swithes between foraging modes, the time scale of mode switches, or the effects of mode switches on feeding rates.

Golden shiners use at least two distinct foraging modes to capture zooplankton. They particulate-feed on large prey (Suffern 1973), locating and attacking individual prey items in a manner similar to that described for the alewife (Janssen 1976a). Golden shiners also pump filter-feed on high densities of small zooplankton by rapidly opening and closing their mouths, sucking in water then forcing it out through their gill rakers. They swim more slowly when filter-feeding compared with particulate-feeding and do not appear to direct movements visually.

In this study, I conducted laboratory experiments to characterize the form of shiner planktivory and mode switching. The first set involved foraging trials with shiners feeding either on *Daphnia* (large prey) or *Bosmina* (small prey) at various densities to determine particulate- and filter-feeding functional responses, respectively. Shiners were also fed a combination of four locally abundant zooplankton species to determine their relative susceptibility to filter-feeding. The final set of exper-

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iments was designed to investigate how readily shiners switch between particulate-feeding on *Daphnia* and filter-feeding on *Bosmina* when both prey types are present simultaneously.

Methods

Golden shiners (*Notemigonus crysoleucas*) were seined from Wintergreen Lake, Kalamazoo County, MI. Fish were sorted into three distinct size classes (mean standard lengths: 58, 80, and 110 ± 1 mm). Examination of scale annuli showed that these size classes corresponded to ages 1+, 2+, and 3+, respectively. Fish were held in 120-L aquaria (10 fish per aquaria). Most fish began feeding in the laboratory within 2 d and were fed daily a mixed diet of *Bosmina*, *Ceriodaphnia*, copepods, and *Daphnia* collected from local lakes for at least 1 mo prior to the beginning of the experiments.

All experiments were conducted in 40-L aquaria (30 cm × 55 cm × 24 cm height) lined on three sides with white paper. The front of each aquarium was left uncovered allowing feeding trials to be videotaped for later slow motion analysis on a Sony beta format system. Light was supplied by two 20-W florescent lamps suspended 1.5 m above each aquarium. Because shiners fed more readily when placed together with other shiners, two fish were used simultaneously in all experimental trials.

Particulate-Feeding Experiments

Daphnia pulex, collected either from local ponds or laboratory cultures, were size-sorted via serial sieves and subsamples measured, dried, and weighed (mean length = 1.1 mm ±0.39 SD; mean weight = 0.0189 mg ±0.0038 SD). A known number of live individuals, not containing ephippia, was put into the tank and allowed to disperse. The lighting conditions and white sided tanks combined with the positive phototactic response of the *Daphnia* to minimize prey clumping in the tanks. For each particulate-feeding trial, two fish of the same size class were placed behind an opaque plexiglas partition in the experimental tank (this area encompassed approximately 10% of the tank volume). The fish were allowed to acclimate for 30 min before the start of the feeding trial. The partition was removed and the shiners were allowed to feed for 5 min or until all *Daphnia* were eaten. Captures were recorded in timed sequence for each fish separately from slow motion replay of the videotapes and these data used for calculating particulate-feeding rates. To help control for depletion effects only prey captures up to 50% prey depletion were used in the analysis presented below. Individual shiners were used for only one trial per day, fed to satiation following the trial and starved for 12 h prior to being used in another trial.

Filter-Feeding Experiments

Bosmina longirostris were collected from Wintergreen Lake and subsamples measured, dried, and weighed (mean length = 0.36 mm ±0.081 SD; mean weight = 0.0008 mg ±0.0002 SD). *Bosmina* collections were combined and mixed in a large fiberglass culture vat and subsamples taken from this vat were counted to determine *Bosmina* density. Buckets of water taken from the vat were then poured into experimental aquaria, then diluted to achieve the various prey densities required for the feeding trials. Densities of 250, 500, and 750 *Bosmina* per litre were used. Two shiners were introduced into the aquaria and allowed to feed. After 5 min the fish were removed from the aquaria and anesthetized with tricane-methane-sulfonate (MS222). Their digestive tracts, including the buccal and gill areas were removed and preserved in 10% buffered formalin. Prey were removed by dissection and rinsing of the digestive tract, and the number of prey ingested was determined by examination under a dissecting microscope. Depletion of *Bosmina* during feeding trials never amounted to greater than 8% of their initial density. Spaces between the base of the gill rakers were measured using an ocular micrometer. Trials were videotaped and the initiation of filter-feeding and the elapsed time until each fish was removed from the tank was recorded. The prey captured divided by the time spent filtering allowed for direct calculation of filter-feeding rates. Buccal volumes were determined for a sample of 20 dead fish by injecting silicone sealant into the buccal cavity, allowing it to cure, and then measuring the volume of the silicone "plug" in a volumetric cylinder.

A second set of experiments was conducted to determine the selectivity of filter-feeding shiners when presented with a combination of different zooplankton species. Experiments were performed as above with the exception that approximately equal densities of calanoid and cyclopoid copepods, *Bosmina*, and *Ceriodaphnia* were offered simultaneously to shiners in aquaria.

Mode Switching Experiments

To determine whether golden shiners switch between filter- and particulate-feeding modes on a short time scale, foraging trials were conducted with both *Daphnia* and *Bosmina* present in aquaria. As in the filter-feeding experiments (described above) two 80-min shiners were allowed to filter-feed on a density of 600 *Bosmina* per litre. However, each 30 s during the experiment a set number of 1.1-mm *Daphnia* was introduced into the tank via 30-cc syringe attached to plastic tubing (5, 10, 15, 20, and 25 *Daphnia* in each successive interval). This method of prey introduction resulted in no visible disruption in the shiners foraging behavior. After 180 s, no more *Daphnia* were introduced and their density decreased. Shiners were removed at 390 s, anesthetized with MS222 and treated as described in the filter-feeding experiments.

The number of *Daphnia* captured during each 30-s interval was determined from slow motion replay of the videotapes. Filter-feeding rates were calculated by counting the number of filtering pumps from slow motion videotape replay during each 30-s interval, then distributing the total number of *Bosmina* in the gut into each time interval in proportion to the number of filtering pumps taken during that interval. This technique assumes that each filter-feeding pump captures the same number of *Bosmina*.

Results

Particulate-Feeding Experiments

Videotape replay of particulate-feeding shows that shiners swam continuously while searching for *Daphnia*. They did not swallow each prey item immediately after its capture, but rather, after 5–10 captures shiners paused and swallowed the accumulated bolus of prey before resuming searching. This swallowing behavior included a flaring of the gills and a coughing motion, followed by opening and closing the mouth several times rapidly. Intercapture intervals were easily recorded from the videotape, averaged, and converted to mean foraging rates (milligrams per second) for each trial. The foraging rates for 80-mm shiners increased asymptotically with increasing

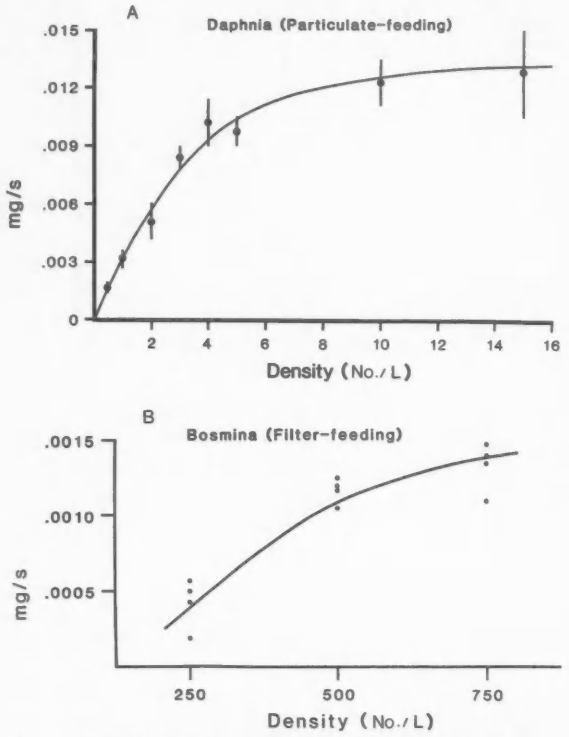

FIG. 1. Particulate-feeding and filter-feeding functional response curves for 80-mm golden shiners; (A) Particulate-feeding rates on *Daphnia* : $Y = 0.012 \cdot (1 - EXP(-0.32 \cdot X))$. Each point is the mean ± 1SE of four trials for four fish. (B) Filter-feeding rates with increasing *Bosmina* density : $Y = 0.0024 \cdot (1 - EXP(-0.00107 \cdot X))$. Each point is the mean for four trials for an individual fish. Regression lines were fit to a negative exponential function using SAS NLIN procedures (SAS 1985).

Daphnia density (Fig. 1a), reaching a maximum of 0.012 mg/s (or approximately one *Daphnia* capture every 1.6 s). Particulate-feeding foraging rates on *Daphnia* increased with shiner standard length (Fig. 2).

Filter-Feeding Experiments

While filter-feeding, shiners moved slowly in a nondirected manner throughout the tank. Filter-feeding shiners would pump-filter for 20–60 s, then pause and swallow the prey collected in their bolus. Pumping behavior appears to be elicited by chemical cues, as evidenced by the fact that shiners would begin pumping when placed into culture water from which all *Bosmina* had been removed by pouring the water through 0.08-mm mesh nytex screening. Shiner filter-feeding capture rates on *Bosmina* increased asymptotically with prey density (Fig. 1b), but decreased with increasing shiner standard length (Fig. 2).

Assuming that *Bosmina* are incapable of active escape from filter-feeding shiners (Janssen 1976b; Drenner et al. 1978), filter-feeding can be viewed as a function of three components (Drenner et al. 1982a; Johnson and Vinyard 1987): (1) pumping rate, (2) the volume of water passed through the gill rakers on each pump, and (3) the efficiency with which the gill rakers retain prey items. Pumping rate decreased with increasing shiner length (Table 1; pumps/s = $66.95 \cdot SL^{-0.59}$, $r^2 = 0.96$, $p < 0.01$). Pumping rates did not appear to change as a function of prey density, although this was not tested explicitly. Buccal volume increased with standard length (Table 1; volume (millilitres) = $9.97 \cdot 10^{-6} \cdot SL^{2.48}$, $r^2 = 0.84$, $p < 0.01$). Filtering rate, i.e. the maximum volume of water that can be passed through the gill rakers per unit time, can be calculated as the product of buccal volume and pumping rate. Filtering rate increases with shiner length, as does the spacing between gill rakers (Table 1; spacing (millimetres) = $0.0024 \cdot SL - 0.0196$, $r^2 = 0.93$, $p < 0.01$). This suggests that the decrease in filter-feeding capture rates with shiner size (Fig. 2) is due to lower retention efficiency of their gill rakers.

When fed roughly equal proportions of various zooplankton species, shiners filter-fed disproportionately on cladocerans as compared with copepods (Fig. 3). Within cladocerans, the larger species (*Ceriodaphnia*) was eaten in greater proportion to its ambient abundance than the smaller species (*Bosmina*). This pattern is most likely due to the greater swimming ability of copepods contributing to their ability to escape suction pressure of filter-feeders (Janssen 1976b; Drenner et al. 1978), and the higher probability that larger prey items will be retained by the gill rakers (Mummert and Drenner 1986).

Mode Switching Experiments

The results of the mode switching experiments are presented in Fig. 4. Each point from left to right represents the mean observed foraging rate for four fish during successive 30-s intervals from the beginning of a trial. The dashed lines show the particulate- and filter-feeding foraging rates predicted for each prey density based upon fish feeding on each prey type alone (see Fig. 1). *Daphnia* density increased as prey were added to the aquaria, and depletion of *Bosmina* was negligible (<5%). During the first 30 s shiners filter-fed exclusively. However, when *Daphnia* were introduced, shiners particulate-fed on *Daphnia* while still filter-feeding between captures. *Daphnia* were pursued and captured when encountered and shiners then resumed filter-feeding. Shiners stopped filter-feeding between captures when *Daphnia* density exceeded 0.75/l, particulate-feeding exclusively until virtually all *Daphnia* were eaten.

Discussion

These experiments demonstrate that (1) golden shiners are capable of both particulate- and filter-feeding, (2) particulate-feeding capture rates increase with shiner size whereas filter-feeding capture rates decrease, (3) cladocerans have a higher susceptibility to filter-feeding than copepods, and (4) golden shiners have the behavioral flexibility to switch between filter-feeding and particulate-feeding on a very short time scale.

Although data on swimming speeds were not collected, it is my strong impression that shiners swam at least twice as fast while feeding on *Daphnia* compared with when filter-feeding on *Bosmina*. Therefore it was expected that by slowing down to filter-feed, particulate-feeding rates would decrease. However, filter-feeding did not appear to interfere with shiner particulate-feeding rates on *Daphnia* (Fig. 4). Although *Daphnia* capture rates in the mode switching experiments are statistically indistinguishable from *Daphnia* capture rates in the particulate-feeding experiments ($p > 0.2$, two-sided t-test for each point in Fig. 4), I believe that this was in part an artifact of the small size of the experimental aquaria. Shiners were capable of detecting *Daphnia* across the width of the aquaria (30 cm) and

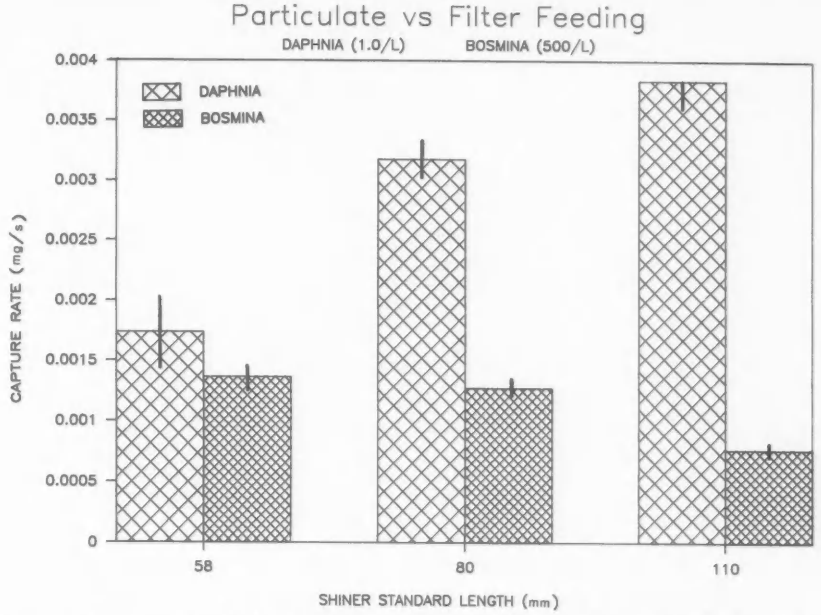

FIG. 2. Comparison of particulate-feeding rate on *Daphnia* (light shading) and filter-feeding rate on *Bosmina* (dark shading) for three different shiner sizes. Data presented are means (± 1SE) for four fish in each size class.

TABLE 1. Buccal volume, pumping rate, filtering rate (calculated as product of buccal volume and pumping rate), and inter-raker spacing for three size classes of golden shiners.

Size	Buccal volume (ml)			Pumping rate (suctions/s)			Filtering rate (ml/s)	Inter-raker space (mm)		
(mm)	Mean	SE	*N*	Mean	SE	*N*		Mean	SE	*N*
58	0.26	0.015	6	6.06	0.13	2	1.58	0.125	0.005	8
80	0.51	0.032	4	4.94	0.08	4	2.52	0.178	0.006	4
110	1.13	0.061	7	4.28	0.17	2	4.84	0.248	0.004	5

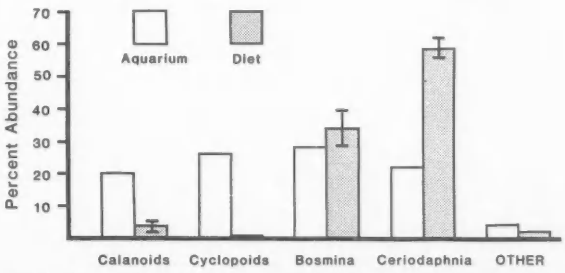

FIG. 3. Comparison of percent numerical abundance of various zooplankton species in aquaria (open bars) and in shiner guts after 5-min feeding trials (shaded bars). Data are means (± 1SE) for four 80-mm shiners.

as such, most of the *Daphnia* in the aquaria were likely within a shiner's visual field (sensu Eggers 1982). In natural lakes however, a slower swimming speed while filter-feeding could significantly decrease the rate of encounters with *Daphnia* and thereby decrease particulate-feeding rates.

Another unexpected result from the mode switching experiments was that shiners did not switch back to filter-feeding as readily as they switched to particulate-feeding; continuing to particulate-feed exclusively until nearly all *Daphnia* were eaten. This hysteresis in favor of particulate-feeding suggests a preference by shiners for *Daphnia*, but this can only be tested properly by conducting the reciprocal experiment (i.e. feeding shiners *Daphnia* then adding *Bosmina* to see when they switched to filter-feeding). I anticipate that filter-feeding will only be exhibited when shiner encounter rates with large zooplankton are very low (e.g. in lakes with very low density of large cladocerans or where their spatial distributions are very patchy).

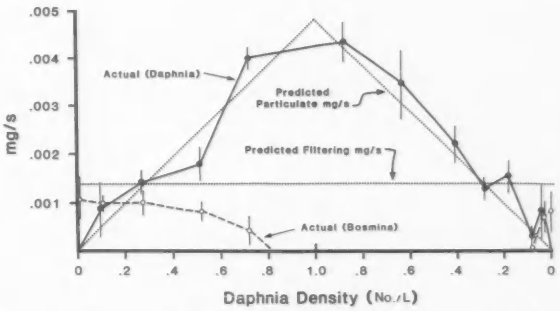

FIG. 4. Comparison of filter-feeding rates (open circles) and particulate-feeding rates (solid circles) across the time course of the mode switching experiments as *Daphnia* density in the aquarium was increased then allowed to deplete. *Bosmina* density was 600/1. Data are means (± 1SE) for four 80-mm golden shiners for each successive 30-s interval in the experiment. Predicted foraging rates were taken from the single-prey experiments (Fig. 1). See text for more details.

The ability to use both particulate and filtering modes has important consequences for shiner foraging ecology. The ability to filter-feed between captures is particularly advantageous when *Daphnia* density is low or when low light levels reduce

encounter rates (Suffern 1973; Hall et al. 1979). The ability to filter-feed on small cladocerans also provides shiners with a resource base unavailable to the other dominant planktivores in local lakes (e.g. bluegill sunfish, *Lepomis macrochirus*) that are apparently capable of visual prey detection only. This behavioral flexibility may allow shiners to persist in lakes not habitable to other planktivores. For example, a partial winterkill in 1979 eliminated the dominant piscivore (largemouth bass) and planktivore (bluegill sunfish) from Wintergreen Lake (Hall and Ehlinger 1989). The winterkill was followed by a rapid increase in the golden shiner population (increasing >10-fold in 4 yr). Concurrent with the rise in shiner numbers was the complete elimination of the large daphnid species from the lake, most probably the direct result of increased planktivory by shiners. *Bosmina* are now very abundant in Wintergreen Lake (often greater than 1000/l in the upper surface waters) and the fact that shiners can filter-feed on this resource allows for their persistence and continued numerical increase in the lake. However, filtering efficiency decreases as shiners grow and this appears to be reflected in a decreased growth rate in adult shiners compared with prewinterkill samples (Hall and Ehlinger 1989). This example illustrates how foraging mode flexibility in fishes can be an important factor affecting both zooplankton and fish community structure.

Acknowledgments

I thank Earl E. Werner and Donald J. Hall for bringing shiner filter-feeding to my attention. An earlier version of this manuscript was improved with the helpful suggestions of Gene Helfman, John Janssen, and Ray Drenner. Ray Drenner also provided valuable advice regarding techniques for measuring buccal volumes and calculating filtering rates. The filtering experiments would not have been possible without the help of Laura Riley. This research was funded, in part by NSF grant BSR-8516841 and BSR-8796176 to E. E. Werner.

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Morphological Deformities in Larvae of *Procladius* Skuse (Diptera: Chironomidae) and Their Biomonitoring Potential[1]

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Warwick, W. F. 1989. Morphological deformities in larvae of *Procladius* Skuse (Diptera: Chironomidae) and their biomonitoring potential. Can. J. Fish. Aquat. Sci. 46: 1255–1270.

Morphological deformities in *Procladius* larvae from sites in eastern and western Canada are described and compared. A framework for categorizing deformities in individual head capsule structures is presented to serve as a basis for indexing the severity of individual deformities and, eventually, to provide a more accurate measure of community response to contaminants. Comparison of morphological responses between *Chironomus* and *Procladius* suggest that *Procladius* is the more tolerant of environmental contaminants. It is proposed to use these differences in response to extend the range over which contaminant concentrations can be monitored.

On décrit et compare des difformités morphologiques des larves de *Procladius* provenant de l'est et de l'ouest du Canada. Un cadre permet de catégoriser les difformités des structures de la capsule céphalique afin d'en évaluer la gravité et, ultimement, de mieux mesurer la réaction de la biocénose aux polluants. La comparaison des réponses morphologiques chez *Chiromus* et *Procladius* indiquerait que ce dernier est plus tolérant face aux pollutants. On propose de mettre à profit ces différences pour allonger la plage des concentrations de polluants pouvant être contrôlées.

Received September 7, 1988
Accepted March 9, 1989
(J9872)

Reçu le 7 septembre 1988
Accepté le 9 mars 1989

Interest in biological monitoring has grown in recent years with the realization that management guidelines based on chemical standards and water quality objectives may not be realistic measures of real world conditions. The contaminant burden borne by freshwater ecosystems has become increasingly complex and it makes eminent sense to use in situ biological communities to monitor the health of these environments. According to Cairns (1986), "even the least effective, crudest ecological methods are superior to pipe and technology based standards for protecting the environment because ecological methods are the only ones that have a feedback loop from the system being protected, based on ecological qualities."

Warwick (1985) proposed using morphological deformities in chironomid larvae as a measure of the long-term chronic toxicity of contaminants in freshwater ecosystems. As a monitoring tool for assessing ambient conditions in the environment, the Chironomidae possess a number of obvious advantages. Among these, the increased number and severity of deformities clearly indicate a response to environmental contamination (Warwick 1985; Kosalwat and Knight 1987). As a first step in developing the tools necessary to interpret these responses, Warwick (1985) developed a numerical index based on deformities in the larval antennae of the genus *Chironomus* Meigen. Warwick and Tisdale (1988) subsequently followed up this work with a framework for assessing the severity of deformities in the other structures of the *Chironomus* head capsule and body. Numerical indices currently are being developed for each of these structures as sufficient data become available and will be included in an over all index of total morphological response. The present study extends this approach to the genus *Procladius* Skuse.

Saether (1970) first reported morphological deformities in *Procladius* larvae from Skaha Lake, British Columbia. His initial work included an illustration of the mandible of a massively deformed larva. This specimen was described in greater detail in Hamilton and Saether (1971) and more recently a photograph was provided in Warwick (1988). Following Saether's early work, a number of workers (Hare and Carter 1976; Warwick 1980a; Krieger 1984; van Urk et al. 1985) listed *Procladius* among deformed larvae, but until recently, few provided details of the deformities themselves. Warwick (1980b) presented detailed illustrations of deformed ligulae from contemporary communities and subfossil assemblages from the Bay of Quinte, Lake Ontario. Tennessen and Gottfried (1983) included similar drawings of deformed ligulae of *Procladius* collected with other deformed Tanypodinae from coal strip-mine ponds and man-made lakes in Marion County, Alabama. Most recently, Warwick and Tisdale (1988) provided detailed descriptions of deformities in ligulae, paraligulae, dorsomenta, and hypopharyngeal pecten in fauna from Tobin Lake, Manitoba.

The purpose of this paper is (1) to expand the data base on morphological deformities in *Procladius* larvae begun in Warwick and Tisdale (1988) and earlier papers, (2) to document deformities in previously undescribed mouthpart structures, (3) to explore the range of severity of these deformities, (4) to compare their distributions in fauna from several different types of aquatic environment and (5) to establish a framework within which deformities in this genus can be evaluated.

[1]Contribution No. NHRI 88056.

TABLE 1. Co-ordinates and possible contaminant status of sites sampled for *Procladius* larvae. Legend: O = organics, M = metals, S = sewage, R = radionuclides.

Site	Longitude (North)	Latitude (West)	Status
St. Lawrence River			
Lac St. Louis Site 1	45 24′23″	73 34′23″	O,M
Lac St. Louis Site 2	45 19′54″	73 55′43″	O,M
Lac St. Louis Site 3	45 19′12″	73 53′35″	O,M
Lac St. Louis Site 4	45 19′07″	73 53′33″	O,M
Lac St. Louis Site 5	45 19′10″	73 53′41″	O,M
Lac St. Louis Site 6	45 19′08″	73 53′23″	O,M
Lac St. Louis Site 7	45 19′15″	73 52′08″	O,M
Lac St. Louis Site 8	45 20′50″	73 48′41″	O,M
Lac St. Louis Site 9	45 25′43″	73 40′26″	O,M
Laprairie basin Site 3	45 27′40″	73 30′04″	O,M,S
Laprairie basin Site 7a	45 24′47″	73 30′02″	O,M,S
Laprairie basin Site 13a	45 24′28″	73 31′31″	O,M,S
Southern Indian Lake-Notigi Reservoir			
Wupaw Bay basin	56 45′19″	99 07′12″	M (Hg)
South Bay basin	56 43′55″	99 05′02″	M (Hg)
Mynarski Lake basin	56 07′32″	99 12′00″	M (Hg)
Rat Lake basin	56 05′31″	99 45′43″	M (Hg)
Last Mountain Lake Site I	50 47′23″	104 56′13″	S,O,M?
Last Mountain Lake Site II	50 59′42″	105 11′58″	O?
Tobin Lake Site 2	53 28′44″	103 50′51″	O,M
Tobin Lake Site 17	53 33′16″	103 28′16″	O,M
Port Hope Harbour (Sites B,C,E)	43 56′36″	78 12′12″	O,M,S,R

Materials and Methods

The specimens used in this study came from a number of different sites in eastern and western Canada (Table 1). The St. Lawrence River specimens were collected during a reconnaissance of a number of sites in the vicinity of Montreal, Quebec. Lac St. Louis Site 1 is located in the ship mooring basin at the head of the St. Catherine locks on the St. Lawrence Seaway. It is dredged, comparatively deep, probably poorly flushed and exposed to considerable contamination. Lac St. Louis Site 2 is located in a position where the Ottawa and St. Lawerence Rivers join and mix upstream of Montreal. Lac St. Louis Sites 3,4,5, and 6 are located in a small bay east of the control structure on the Beauharnois Canal immediately off-shore from the factory complex around Beauharnois, Quebec. Lac St. Louis Site 7 is located off Beauharnois to the east of the mouth of the St. Louis River. Lac St. Louis Site 8 is located further east in the bay formed by the island complex around Iles de la Paix. This area is largely protected from the main flow of the St. Lawrence River, although some exchange of water still does occur. Lac St. Louis Site 9 is located at the upstream entrance to the Lachine Canal. This area is a major small boat harbour, itself probably an immediate source of contaminants.

The Laprairie basin sites are located within the confines of the St. Lawrence Seaway dike system and thus are isolated from the main flow of the river. The shallow waters within the dike system are stagnant and highly eutrophied by untreated sewage from Laprairie, Quebec. From Laprairie Site 3 near the Champlain Bridge, conditions deteriorate steadily westward through Site 7a to Site 13a off-shore from the Laprairie sewage treatment facility.

The southern Indian Lake-Notigi Reservoir specimens were collected from a number of sites experiencing differing degrees of flooding, bank erosion, and mercury contamination (W. F. Warwick, unpubl. data). In Southern Indian Lake, the sediments of oligotrophic Wupaw and South bays are both highly mineralized. According to Shepard's (1954) classification of particle-size distributions, the sediments of the former are classed as silty–clay while the latter are clayey–silts. The difference most likely is a function of the greater mixing of eroded shoreline materials in South Bay. In Notigi Reservoir, mineral materials eroded by rising water levels appear to have modified the composition of the former lake sediments at the Rat Lake site to a certain degree. Classed as silty–clay, there is a patina of very fine clay-sized material intermixed with these sediments which undoubtedly are eroded materials redeposited in a deep-water environment. The sediments at the Mynarski Lake site are mainly clay-sized materials. These sediments have been affected less by the importation of eroded mineral sediments and are highly organic in nature. These latter sites probably are mildly eutrophic. The degree to which fauna have been exposed to increased levels of mercury has been correlated with the extent of areal flooding in the different basins of the reservoir (Bodaly et al. 1984a,b).

The Last Mountain Lake specimens were collected from sites located approximately midway along the east–west and north–south arms of the lake (Warwick 1985). Last Mountain Lake Site I is shallow, eutrophic and contaminated by flood waters from the Qu'Appelle River system that are shunted back up into the lake to alleviate downstream flooding in the spring (Warwick 1988). Last Mountain Site II is located ca. 32 km further north. To what extent it is affected by contaminants from the Qu'Appelle system is difficult to estimate, but circulation patterns probably confine contaminants from this source to the lower reaches of the lake. Contaminant residues from surrounding agricultural areas are likely present in the sediments of both sites.

The Tobin Lake specimens come from single sites in each of the two basins forming the Tobin Lake reservoir (Warwick and Tisdale 1988). Tobin Lake Site 2 is located near the inflow

of the Saskatchewan River and is directly exposed to contaminant and sedimentary materials from throughout the greater prairie region of Canada. Tobin Lake Site 17 is located in a backwater area of the reservoir surrounded by agricultural lands. In contrast, this latter site receives only a fraction of the sediment and contaminant loadings impacting the former site (Warwick 1988). Both sites are probably mildly eutrophic.

The Port Hope specimens have been pooled from three sites (B,C,E) to provide a representative sample of the fauna in the inner harbour basin (Warwick 1988). The harbour is used as an anchorage area for a yacht club and a receiving basin for wastes from a uranium refining plant. It is heavily contaminated with radionuclide wastes, cooling water, and heavy metals (Hart et al. 1986; Warwick et al. 1987).

Benthic samples were collected using a tall 232-cm^2 Ekman dredge. The number of dredges collected at each site varied depending on population densities and work load involved in collection. Each sample was sieved through a 200-μm mesh sieve net of Nitex nylon. Material retained in the sieve was preserved in 70% ethanol, stained with Rose Bengal and sorted. All benthos were sorted into individual taxa, enumerated, and preserved in fresh 70% ethanol for future analysis.

Preserved chironomid larvae were prepared and mounted in Canada balsam according to methods described in Warwick (1980a,b, 1985). These methods are particularly adapted for examining fine morphological detail at higher microscopic powers. No attempt was made to identify larvae to species because of the problems involved in the identification of chironomid immatures (cf. Lindeberg and Wiederholm 1979).

The framework used to categorize deformities in the structures of the head capsule is outlined in Table 2.

As in Warwick and Tisdale (1988), the term frequency (sensu prevalence; Margolis et al. 1982) is defined as the ratio of the number of actual to possible occurrences expressed as percent. This replaces the term incidence used previously in Warwick (1985).

Results

Chironomid Community Comparisons

The communities from which the *Procladius* larvae were extracted for this study varied considerably in composition (Table 3) and environment. *Procladius* formed the dominant component of the fauna in all Lac St. Louis sites with only Sites 7 and 8 having any other single genera in appreciable numbers. At Site 7, *Cryptochironomus* was almost as abundant as *Procladius* whereas at Site 8, the next most abundant genus, *Cryptotendipes*, was roughly half as abundant as *Procladius*. The only other site to have a dominant *Procladius* fauna was Last Mountain Lake Site I.

Chironomus formed the dominant component of the remaining sites, with the exception of Laprairie basin Site 13a and the Wupaw Bay basin. *Cryptochironomus* was the most

TABLE 2. Provisional framework for categorizing deformities in head capsule structures of *Procladius* larvae.

Structure	Category
Antenna	a) basic classification categories
	i) loss of genuine segments
	ii) presence of questionable segments
	b) reduction in antennal length
	c) displacement, loss or addition of ring organs
	d) apical fusing of basal segments
	e) displacement, loss or addition of sensory organs
	f) presence of structures or unknown homology
	i) major structures
	ii) minor structures
Ligula	a) number of teeth
	b) modifications to outer, larger teeth
	c) overlapping of teeth
	d) presence of accessory teeth
	e) presence of forked teeth
	f) fusing of ligula and paraligula
	g) massive disorganization
	h) asymmetry
Paraligula	a) superpositioning of teeth
	b) asymmetry
	c) reduction in size of teeth
	d) absence of teeth
Mandible	a) number of subapical teeth
	b) conformation of teeth
	c) presence, form and conformation of spurs, setae and pecten
	d) general configuration of mandible
Dorsomentum	a) number of teeth
	b) configuration of individual teeth
	c) overlapping of teeth
	d) massive disorganization of teeth
Hypopharyngeal pecten	a) presence of bifid spines

TABLE 3. Sampling data, population statistics, and frequency of deformities in *Procladius* spp. larvae and other larvae of the family Chironomidae; in terms of total chironomid community.

	Depth of water (m)	Number of samples	Total number of chironomid larvae examined	Population density (Larvae·m^{-2})	Number of taxa	*Procladius* number	*Procladius* % deformed	Other genera % deformed	Family chironomidae % deformed
St. Lawrence River									
Lac St. Louis Site 1	ca.10.	1	46	1981	10	19	4.3	15.3	19.6
Lac St. Louis Site 2	7.6	5	253	2178	11	190	7.5	2.4	9.9
Lac St. Louis Site 3	—	1	71	3057	8	52	8.5	0.0	8.5
Lac St. Louis Site 4	8.3	5	368	3169	10	281	8.4	1.4	9.8
Lac St. Louis Site 5	6.2	5	492	4237	20	391	9.6	1.00	10.6
Lac St. Louis Site 6	12.5	5	441	3798	17	324	7.9	1.2	9.1
Lac St. Louis Site 7	4.0	5	369	3178	22	142	3.5	3.0	6.5
Lac St. Louis Site 8	5.2	5	704	6062	25	234	5.0	0.3	5.3
Lac St. Louis Site 9	5.6	4	47	506	9	30	8.5	0.0	8.5
LaPrairie basin Site 3	1.7	4	62	667	8	11	8.1	25.8	33.9
LaPrairie basin Site 7a	2.6	4	17	183	5	4	5.9	47.0	52.9
LaPrairie basin Site 13a	2.1	4	10	108	3	1	0.0	20.0	20.0
Southern Indian Lake-Notigi Reservoir									
Wupaw Bay basin	4.5	5	340	2928	25	26	0.3	0.6	0.9
South Bay basin	5.5	5	156	1343	13	65	1.3	0.0	1.3
Mynarski Lake basin	16.0	20	734	1580	6	76	1.0	17.3	18.3
Rat Lake basin (spring)	16.0	20	890	7380	7	6	0.0	23.7	23.7
Rat lake basin (summer)	15.0	20	1260	2713	7	11	0.1	63.2	63.3
Last Mountain Lake Site I	3.9	2	1174	25 274	7	619	0.8	3.8	4.6
Last Mountain Lake Site II	23.1	2	935	20 119	7	93	0.3	9.9	10.2
Tobin Lake Site 2	10.1	3	1010	14 495	22	344	1.4	20.5	21.9
Tobin Lake Site 17	16.0	3	309	4435	11	97	0.7	16.8	17.5
Port Hope Harbour (Sites B,C,E)	3.7	—	174	—	11	66	6.9	25.3	32.2

abundant genus at Laprairie basin Site 13a whereas *Pagastiella* and members of the Tanytarsini formed the most abundant components of the Wupaw Bay community. Members of the sediphilic Tanytarsini community were also very common in South Bay as would be expected in a zone subjected to the rapid accumulation of erosion sediments, but in terms of abundance, *Procladius* was the single most important genus.

The effects of gross pollution within the Laprairie basin were evident (Table 3) in the decline in population densities from Site 3 to Site 13a. Chironomid larvae were absent from sites closer inshore between Site 13a and the Laprairie sewage treatment facility.

The impact of deteriorating conditions within the Laprairie basin also influenced the interpretation of the over all frequency of deformities presented in Table 3. Although the highest frequency given is from Rat Lake, Laprairie basin Site 13a probably was the more seriously affected of the two sites. The frequency given for Site 13a (20.0%) is based only on a remnant fauna. The trend shown in the increasing frequency between Sites 3 and 7a (33.9 to 52.9%) probably would have continued at Site 13a if the effects of larval mortality had not over-ridden morphological response.

Within the genus *Procladius* itself, the highest frequency of deformation was recorded at Lac St. Louis Site 5 located just offshore from the factory complex at Beauharnois. The frequency of deformities (9.6%) at this site is more than 12 times higher than its most comparable site, Last Mountain Lake Site I. The latter site does receive contaminants from the Qu'Appelle River system, but it is not likely as contaminated as the St. Lawrence River site. The frequency of deformities at most of the Lac St. Louis sites in the immediate vicinity of the factory complex (Sites 3,4,5, and 6) and the entrance to the Lachine canal (Site 9) are roughly comparable with, if not slightly higher than, the frequency of deformities (6.9%) in the *Procladius* fauna from Port Hope Harbour.

Description of Morphological Deformities in *Procladius* Larvae

1. Normal and deformed antennae

The antenna of *Procladius* has four segments (Fig. 1a), the basal segment of which is considerably longer than the total length of the apical segments. The ring organ is located apically 0.75× from the base of the basal segment. No basiconic sensillum appears to be present. The antennal blade and conjoined accessory blade are located on the apex of the basal segment (enlargement Fig. 1b). Also located in this position are two tiny, dark-coloured, crescent-shaped structures similar to the structures located between the third and fourth segments on *Chironomus* antennae; these structures on *Procladius* antennae may represent peg sensillae as in *Chironomus* (Warwick and Tisdale 1988).

There are three structures located at the apex of the second segment (enlargement Fig. 1b), the larger of which is similar in structure to the style on *Chironomus* antennae. These structures probably also serve as mechanoreceptors (Warwick and Tisdale 1988). The two smaller structures (referred to as sen-

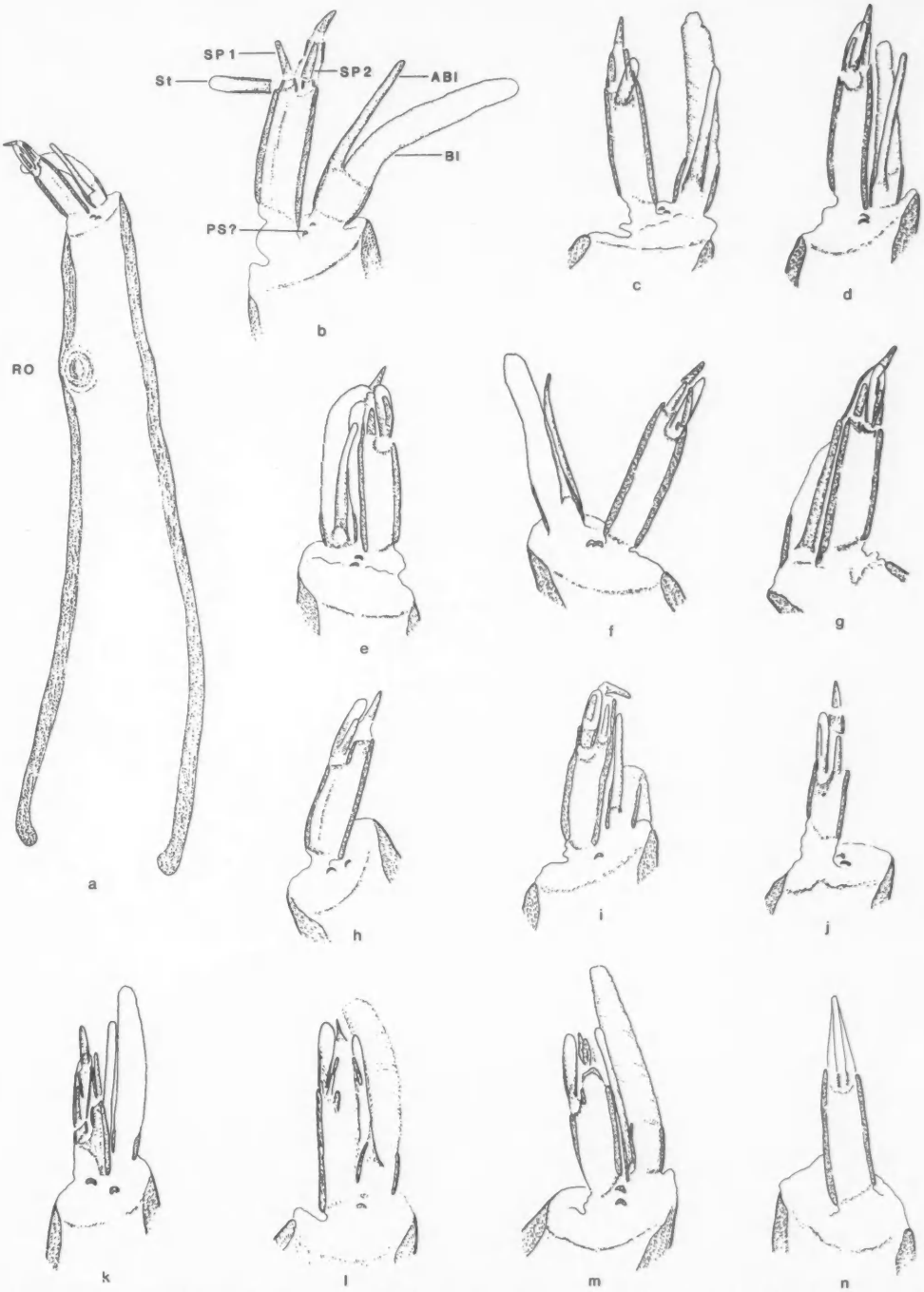

FIG. 1. Deformities in antennae of *Procladius* larvae: (a) normal, ((b) with apical segments enlarged), (c–n) deformed antennae. Abbreviations: blade (B1), accessory blade (AB1), style (St), peg sensillum (PS), ring organ (RO), and sensory pegs (SP).

sory pegs SP_1 and SP_2 in Roback 1980) appear tube-shaped and heavy-walled. Each is mounted on an individual pedestal rather than directly on the articulating membrane linking Segments II and III. This would seem to rule out the mechanoreceptor role accorded the organs of Lauterborn in *Chironomus*. Their structure is more similar to thick-walled chemoreceptors and may serve in direct contact reception of stronger chemical stimuli (Warwick 1985).

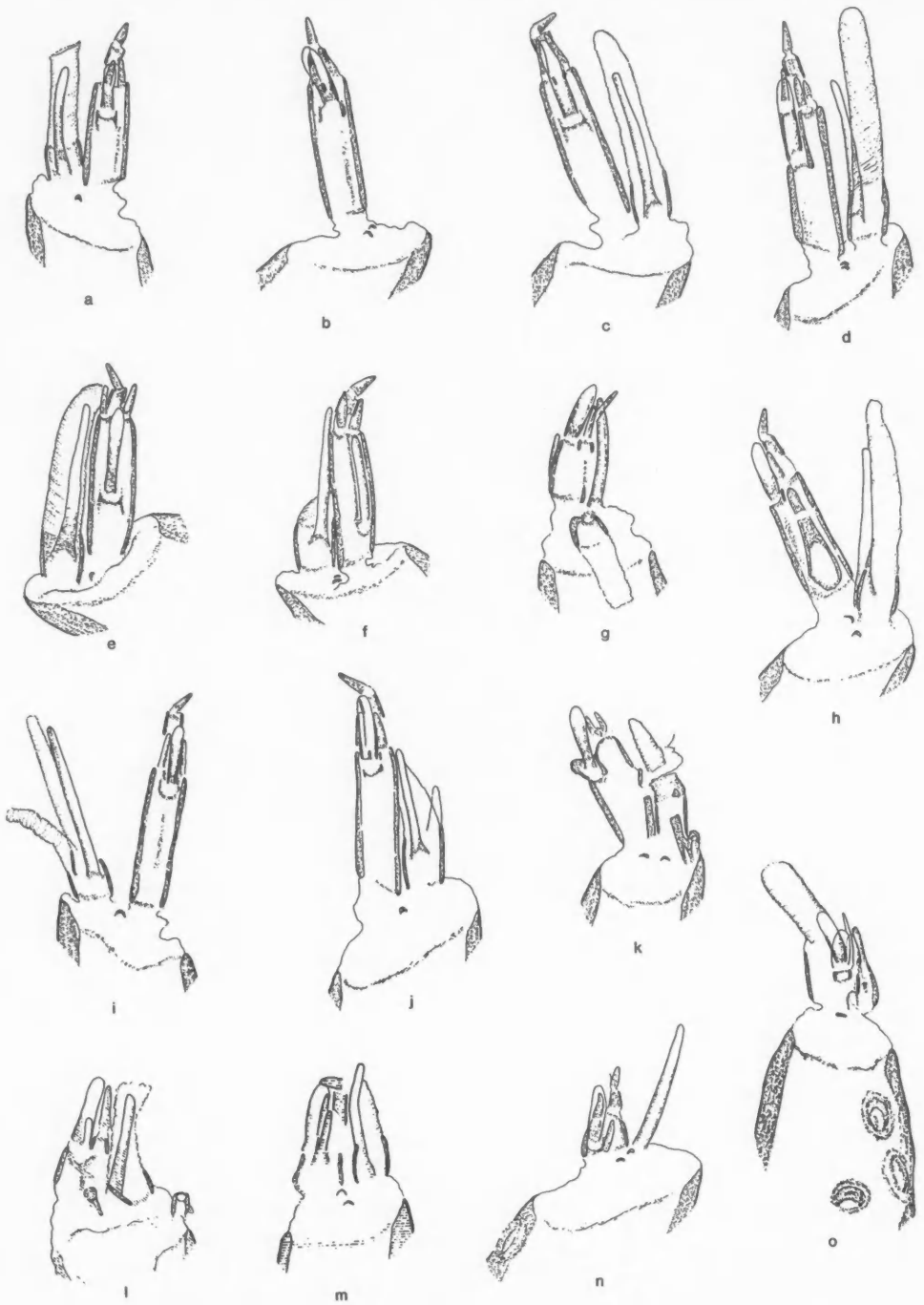

FIG. 2. Deformities in antennae of *Procladius* larvae: (a–o) deformed antennae.

Antennal deformities in *Procladius* involved (a) the loss of individual segments or the presence of questionable segments including subapically-mounted terminal segments, incompletely sclerotized segment walls, clustered apical segments, reduced or stunted segments, fused segments, and multiple questionable segments, (b) the reduction in length of segments, (c) the displacement, loss, or addition of ring organs, (e) the displacement, loss, or addition of sensory organs, and (f) the

presence of structures of unknown homology. No deformities analogous to (d), the apical fusing in *Chironomus*, were noted. Based on the present data, the category dealing with the displacement or loss of the ring organ has now been modified from Warwick (1985) to include the presence of additional ring organs. Similarly, the displacement of sensory organs now includes the loss of these organs.

Individual antennae lacking one or more apical segments are shown in Figs. 1 and 2. The penultimate segment (Segment III) is lacking in the antennae in Figs. 1c–f while Segments III and IV and Segments II, III, and IV, are lacking in Figs. 2l and 2o, respectively. These antennae represent segment complements of three, two?, and one, respectively, according to Warwick (1985). Antennae represented solely by the basal segment which, in *Chironomus*, often are much reduced in length and completely fused distally by a heavy chitinous wall, have not been found as yet in this genus.

A number of antennae in which the loss of segments is questionable were found in *Procladius*. In the antennae in Figs. 1g–i, Segments II and III are partially fused to give a total complement of three? segments. This is based on two unambiguous segments and one? questionable segment (fused Segments II and III) according to Warwick (1985). In Fig. 1j, the apical segments, particularly Segment II, are much reduced in length compared with those on the companion antenna and would be regarded as questionable segments. The segments of the antenna in Fig. 1k are reduced in length, but the wall of Segment II also is incompletely sclerotized. Similarly, the wall of Segment II on the antenna in Fig. 1l is only partially sclerotized, but Segment IV is also atrophied and sensory pegs SP_1 and SP_2 are reduced in size and displaced from their usual position at the apex of Segment II. In Fig. 1m, Segment III, itself irregular and much reduced in size, is crowned by a cluster of three 'segments'. These may represent the apical segment and manifestations of the sensory pegs SP_1 and SP_2 since both are missing from their normal position at the apex of Segment II. However, these additional Segments do not have the tubular construction of sensory pegs and more likely represent clustered apical segments. In Fig. 1n, apical segments III and IV have been replaced by one or two sheath-like, transparent structures which lie behind the one remaining pedestal-mounted sensory peg. The two tiny, dark-coloured, crescent-shaped structures lying on the articulating membrane between Segments I and II also appear to be missing.

The displacement of sensory organs is shown in the progressive displacement proximally of the style in Figs. 2a–f. The antenna of Fig. 2d is interesting because, in addition, it has three sensory pegs rather than the normal complement of two. In Fig. 2g, one sensory peg has been displaced laterally along side Segment II which itself is much reduced in length and incompletely sclerotized, and the accompanying blade/accessory blade complex, normally conjoined, is separated into individual structures. The antenna in Fig. 2h has an enlarged structure displaced laterally along side Segment II which probably represents an aberrant sensory peg as there already is only one normal example properly located between Segments II and III.

The blade/accessory blade complex on the antenna in Fig. 2i has two blades rather than one whereas the antenna in Fig. 2j has two accessory blades rather than one. The antenna in Fig. 2k also displays deformities in the blade/accessory blade complex with a second rudimentary complex appearing at the apex of basal Segment I just below the base of the fully developed organ complex. Segments II and III are also partially fused on this antenna, there are at least two segments clustered at the apex of the antenna and there is an unknown structure budding off the antenna near the base of the style.

The remaining antennae are all seriously reduced in length and display a number of aberrant features. The antenna in Fig. 2l only has one apical segment and this is only partially sclerotized. The second sensory peg at the apex of this segment does not appear to be fully developed and there is a structure of unknown homology located at the apex of basal Segment I. All three apical segments are present on the antenna in Fig. 2m, but each is greatly reduced in length and Segment II is only partially sclerotized. The three apical segments of the antenna in Fig. 2n are similarly reduced in length. The blade/accessory blade complex in this example is represented only by the filamentous part of the accessory blade; the heavily sclerotized basal portion of the structure is lacking. In Fig. 2o, the style and sensory pegs are mounted laterally along side the base of the blade/accessory blade complex; the apical segments on which these structures normally are located are lacking. The basal segment in this example is most unusual in that it has three ring organs clustered near its apex. Previous experience with *Chironomus* has been that the ring organ usually is displaced or lost; antennae with additional ring organs like this have not yet been reported in this genus. The basal segment in this example also is greatly reduced in length.

2. Normal and deformed ligulae

The ligula of *Procladius* is a flexibly-mounted stabbing mouthpart which replaces the mentum of other chironomid subfamilies. It normally has five dark brown to black teeth arranged in a concave row (Fig. 3a). The teeth are hollow and usually wear or break unevenly (Fig. 3j). The ligula is flanked on either side by leaf-shaped paraligulae which will be discussed below independently.

Deformities in the ligula usually concern (a) the number of teeth, (b) modifications to the outer, larger teeth, (c) overlapping of teeth, (d) the presence of accessory teeth, (e) the presence of forked teeth, (f) the fusing of one or more paraligula with the ligula, (g) massive disorganization, and (h) asymmetry in the ligula.

The loss of one tooth on the ligula (Figs. 3c,d) represents one of the most common types of deformed ligulae, but ligulae with only three teeth have also been found (Fig. 3b). Ligula are also known with five asymmetrical teeth (Fig. 3e), seven teeth, the two extra of which are tiny teeth located between the two larger outer teeth (Fig. 3f), and six teeth, the extra teeth of which may (Fig. 3h) or may not (Fig. 3g,i) be uniform in size. V. Pettigrove (Rural Water Commission of Victoria, Armadale, Victoria, Australia, pers. comm.) has found ligulae bearing as many as nine teeth in *Procladius* larvae from the Murray River and its tributaries.

The ligulae in Figs. 3k–m show the progressive reduction in size of the larger outer tooth of the ligula. Both V. Pettigrove (pers. comm.) and Tennessen and Gottfried (1983) found ligulae of similar configuration. The ligula in Fig. 3n is different in that the reduced outer tooth overlaps the intermediate inner tooth.

The ligulae may bear one (Fig. 3o) or two (Fig. 4a) small accessory teeth on the outer margin of the ligula at the point where the larger teeth flare out from the main body. These may be difficult to distinguish from ligulae in which the outer tooth is reduced in extremis, but accessory teeth usually appear somewhat lower on the side of the ligula. Similarly it may be difficult

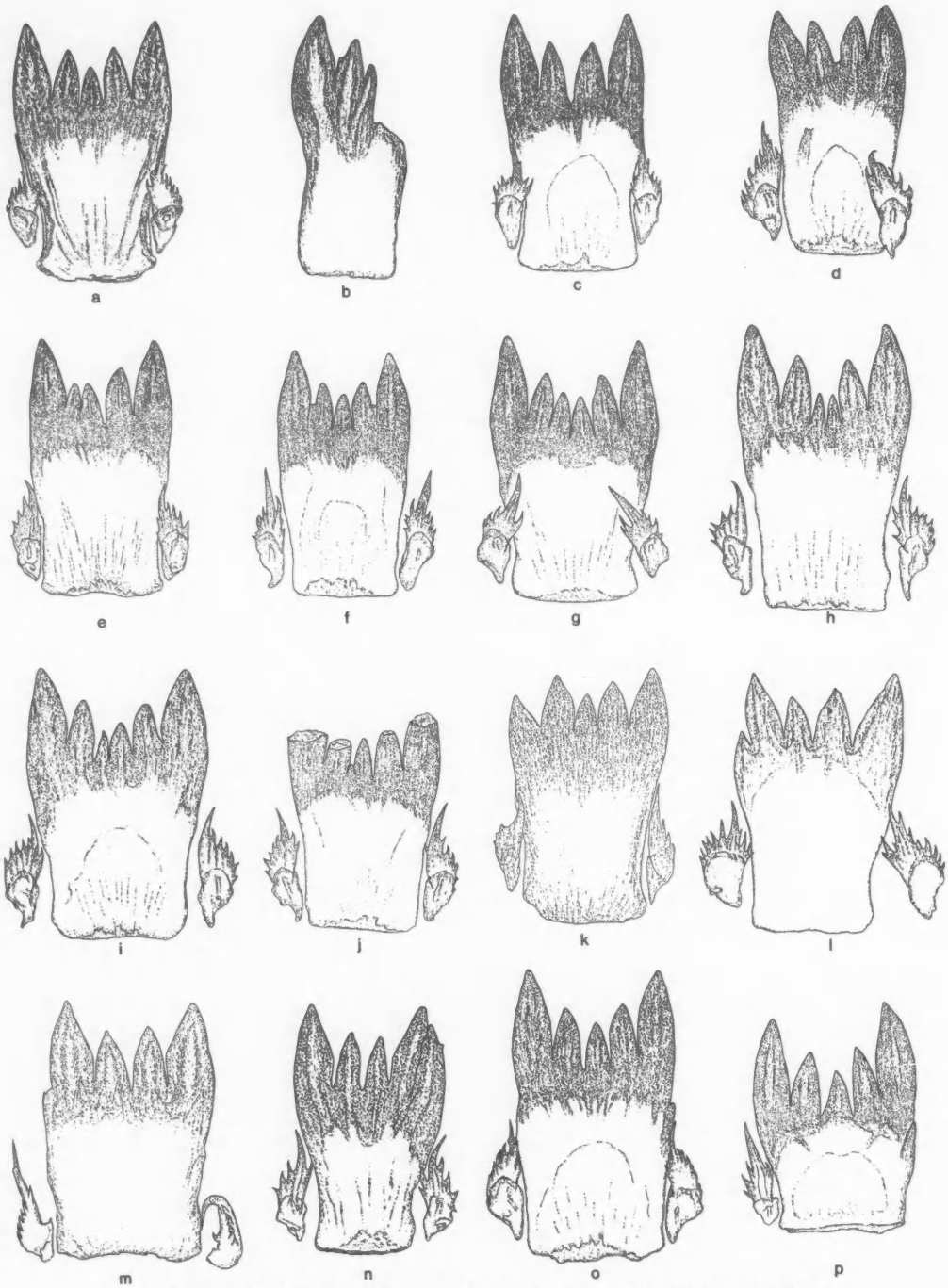

FIG. 3. Deformities in ligulae of *Procladius* larvae: (a) normal and (b–p) deformed ligulae.

to distinguish these from fused paraligula which bear no teeth. These may involve one (Fig. 3p) or both (Figs. 4b,c) paraligula and can usually be differentiated from accessory teeth by thinning of the chitin (apparent as a color separation) at the point of fusion and by the absence of one (Fig. 3p) or both (Figs. 4b,c) paraligula from their flanking position.

Different types of fusing between the ligula and paraligula can occur; the paraligula may appear to be mounted on a pedestal (Figs. 4d–g) or grafted to the side of the ligula (Figs. 4h,i).

Forking can occur in any one or more of the teeth from the

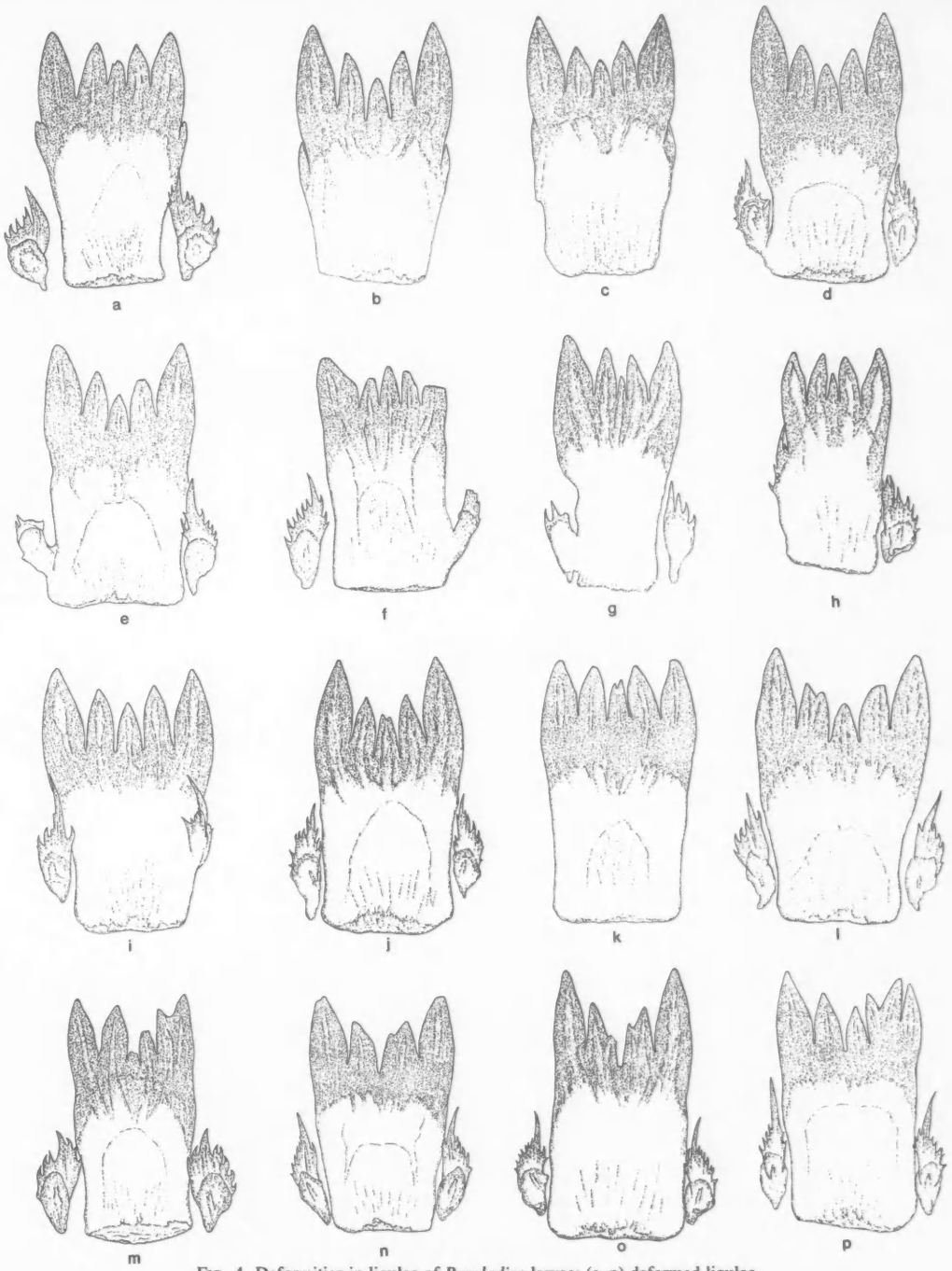

FIG. 4. Deformities in ligulae of *Procladius* larvae: (a–p) deformed ligulae.

small central tooth (Figs. 4j,k) to intermediate (Fig. 4l) and outer (Fig. 4m) teeth. V. Pettigrove (pers. comm.) similarly found examples of forked teeth in fauna from the Murray River and its tributaries.

Ligulae bearing combinations of these basic types of deformities are illustrated: ligula with only four teeth, one (Fig. 4n) or two (Fig. 4o) of which may be forked; ligula with five teeth, one of which is forked and the outer one reduced (Fig. 4p), the central tooth and both intermediate teeth of which may be forked and both outer teeth reduced (Fig. 5a), one to which the para-

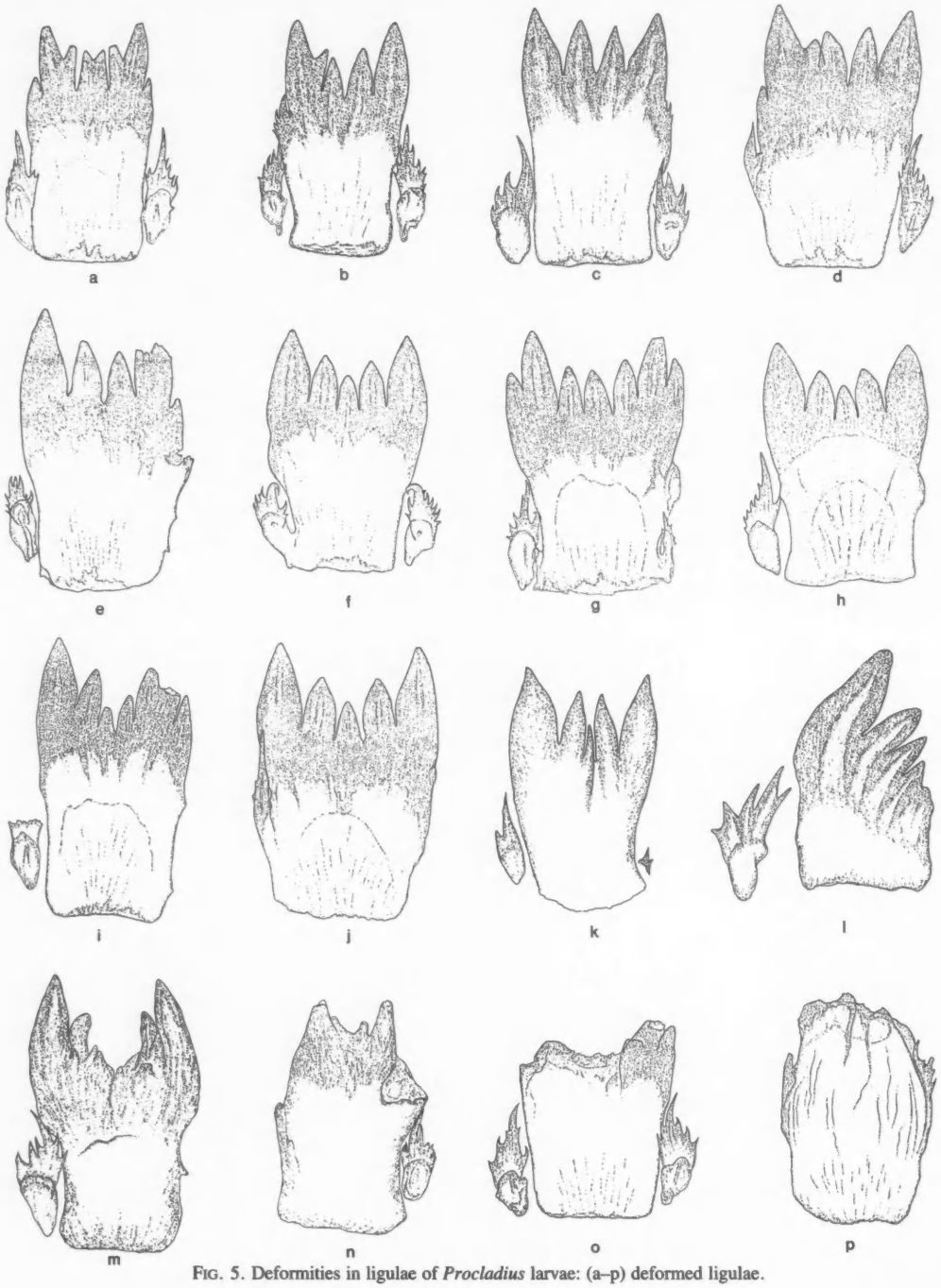

FIG. 5. Deformities in ligulae of *Procladius* larvae: (a–p) deformed ligulae.

ligula may be fused (Figs. 5f,h,j), one to which the paraligula may be fused (Figs. 5g,j) and possessing one (Fig. 5i) or two (Fig. 5g) accessory teeth; and ligula with four teeth, one of which may be forked (Figs. 5b,d,e), one to which a paraligula (Fig. 5d) or a third (extra) paraligula (Figs. 5b,c) may be fused, one forked tooth of which overlaps another tooth (Fig. 5d) or which possess accessory teeth (Figs. 5d,e).

Two examples of atrophied or misshapen ligulae have been found in early instar larvae. The ligula in Fig. 5k, along with its accompanying paraligula, is withered and asymmetrical

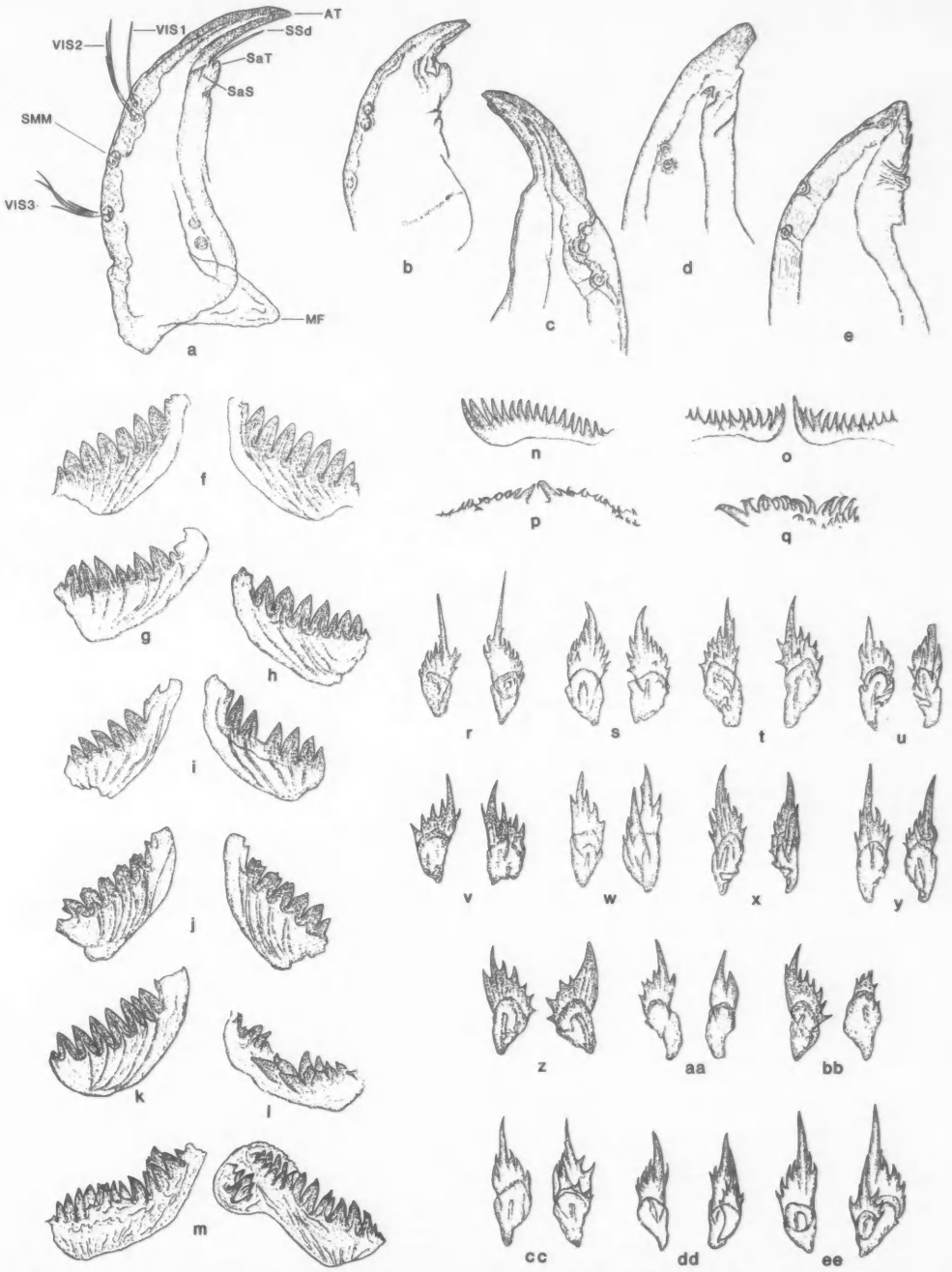

FIG. 6. Deformities in mandibles, dorsomenta, hypopharyngeal pecten, and paraligulae of *Procladius* larvae: (a) normal and (b–e) deformed mandibles; (f) normal and (g–m) deformed dorsomenta; (n) normal and (o–q) deformed hypopharyngeal pecten and (r) normal and (s–ee) deformed paraligula. Abbreviations: apical tooth (AT), subapical tooth (SaT), seta subdentalis (SSd), subapical spur (SaS), ventrolateral setae (VlS), and sensillum minisculum (SMM).

while that in Fig. 5l is strongly warped to the left side. Both specimens have five teeth.

Massively deformed ligulae are shown in Figs. 5m–p. The ligula in Fig. 5m bears nine teeth warped into a semi-circular arrangement. The protrusion on the lower left margin may or may not be the remnant of the missing paraligula. The ligulae in Figs. 5n,o are difficult to describe verbally, but the blunted-off ligula in Fig. 5p appears at least to have paraligula fused to

either side. V. Pettigrove (pers. comm.) found a massively deformed ligula simlar to that illustrated in Fig. 6p.

3. Normal and deformed paraligulae

The paraligulae are leaf-shaped structures (Fig. 6r) located at the base and on either side of the ligula (Fig. 3a). They have a variable number of teeth, most of which occur along the outer margin of the paraligula. The function of the paraligula is not known.

Deformities in the paraligula include superimposed teeth ranging from small (Figs. 6s,t) to increasingly larger single spurs (Figs. 6u–x) and more complex spurs (Figs. 6y,cc) which increasingly resemble (Figs. 6dd,ee) miniature paraligula in themselves. Other paraligulae may be asymmetrical with their counterpart (Fig. 6z), lacking in teeth (Fig. 6aa) or have teeth that are reduced in size (Fig. 6bb).

4. Normal and deformed mandibles

The mandibles of *Procladius* larvae are more slender than their *Chironomus* counterparts with a single long apical tooth and single subapical tooth (Fig. 6a). There is a single subapical spur at the level of the subapical tooth and immediately below the origin of the seta subdentalis, three sets of ventrolateral setae and a single sensillum minisculum on the outer margin and a strong mandibular flange. Terminology is after Cranston and Reiss (1983) and Saether (1980).

Although simpler than *Chironomus*, deformities in *Procladius* mandibles generally involve the same features. These may include apical teeth that are reduced in length (Figs. 6b,d), truncated (Fig. 6e) or atrophied (Fig. 6c), subapical teeth that are absent (Fig. 6c), reduced in size (Fig. 6d) or have one (Fig. 6b) or more (Fig. 6e) additional teeth, and subapical spurs and setae lacking (Figs. 6c,d). The specimen in Fig. 6e also is noteworthy for its greatly thickened chitinous wall.

5. Normal and deformed dorsomenta

The dorsomentum, the dorsal plate of the double walled mentum (Fig. 6f; hypopharyngeal combs sensu Warwick and Tisdale 1988), normally bears a set of triangular shaped dorsomental teeth. These vary in number from three to nine on each side of the mentum depending on the species and larval instar. The dorsomental teeth in Fig. 6f are from a fourth instar larva and have seven and eight teeth on the left and right sides, respectively.

Deformities in the dorsomenta include teeth of reduced size (Figs. 6g,h), reduced numbers of teeth (Fig. 6i), bifid teeth or teeth crowned with smaller subteeth or points (Fig. 6j), overlapping teeth (Fig. 6k) or teeth which are increasingly disorganized (Figs. 6l–m).

6. Normal and deformed hypopharyngeal pecten

The hypopharyngeal pecten are a transverse row of (generally) 8–16 simple teeth or spines (Fig. 6n) on each side mediobasal on the hypopharynx (Saether 1980). The pecten in Figs. 6o–q all have one or more bifid teeth in their complement.

Occurrence of Characteristic Deformities

Prementohypopharyngeal complex

Except in the dorsomentum, the highest frequencies of deformities within the prementohypopharyngeal complex were recorded from stagnant-water sites in the Laprairie basin and Lac St. Louis Site 1 — the ship-mooring basin at the head of the St. Catherine locks (Table 4). *Procladius* larvae from Laprairie basin, Sites 3 and 7a showed the highest frequencies of deformities in the ligula. Although the Site 7a datum is based on a very small sample (four), the trend between the two sites is consistent with the observation that bottom conditions deteriorate westward towards the Laprairie sewage treatment facility (Saether 1979).

Larvae from Site 3 also showed the highest frequency of fusing between the ligula and paraligula and deformation in the paraligula, and the second highest frequency of deformities in the hypopharyngeal pecten. The frequency of fused ligula/paraligula was equally high in the Rat Lake fauna, but this datum is based on a single deformed specimen from a comparatively rare component (0.9%) of the chironomid community. Without supporting data, the significance of this datum is more difficult to assess than the Laprairie data. Larvae from Lac St. Louis Site 1 had the highest frequency of deformities in the hypopharyngeal pecten and the second highest frequency of fused ligulae and paraligulae.

The frequencies of deformities in the dorsomentum and dorsomental teeth were essentially equivalent (Table 4) in fauna from the South Bay basin of Southern Indian Lake and from Port Hope Harbour. While the sizes of sample were directly comparable, the deformities in the Port Hope larvae were considerably more severe. The South Bay example featured a gap midway along the base of the dorsomentum where a single tooth possibly failed to develop. In contrast, the left side of the dorsomentum was misshapen in the Port Hope larva and both sides of the dorsomentum had numerous additional teeth, many of which were overlapping (Fig. 6m). The example from Tobin Lake Site 2 (Fig. 6l) was also seriously deformed.

The frequencies of deformities in the four structures of the prementohypopharyngeal complex were some 5–10× higher in the fauna from the two stagnant-water sites than in the Port Hope Harbour fauna. Only in those deformities involving fusion of the liqula and paraligula did frequencies approach parity. Deformities in these structures also were more routinely present (Table 4) in the fauna from the Lac St. Louis sites than in either the Southern Indian Lake-Notigi Reservoir sites or the Last Mountain Lake and Tobin Lake sites.

Antennae and mandibles

Procladius larvae from Port Hope Harbour had the highest frequency of deformities in antennae (Table 4). In comparison, the frequency of antennal deformities in Sites 4 and 8, the highest frequencies in the Lac St. Louis series, was less than half as high as in Port Hope Harbour. However, if *Procladius* antennae respond in the same manner as those in *Chironomus*, interpretation of the relative degree of contamination of the two sites will depend on which side of the quantal dose–response curve the respective datum points lie (Warwick 1988). If these points prove to lie on the right side rather than the left side of the bell-shaped distribution curve, then Sites 4 and 8 would be the more highly contaminated sites.

Deformities in the mandible of *Procladius* were quite rare, but when they did occur they did seem relatively severe. Although not illustrated, the example from the Mynarski Lake basin lacked any vestige of apical teeth and was completely fused over. Of those illustrated in Fig. 6, all came from sites in Lac St. Louis.

Single and multiple deformities

The distribution of single and multiple deformities (Table 5) is interesting in that the only triple deformities were grouped in those sites from Lac St. Louis located immediately offshore

TABLE 4. Frequency (%) of types of deformities in *Procladius* spp. larvae; in terms of the *Procladius* component of the chironomid community.

	Ligula		Ligula/ paraligula		Paraligula		Hypo-pharyngeal pecten		Dorsomentum		Antennae		Mandibles	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent
St. Lawrence River														
Lac St. Louis Site 1	—	—	1	5.3	—	—	2	10.5	—	—	—	—	—	—
Lac St. Louis Site 2	11	5.8	2	1.1	1	0.5	4	2.1	—	—	2	1.1	—	—
Lac St. Louis Site 3	2	3.8	—	—	—	—	4	7.7	—	—	—	—	—	—
Lac St. Louis Site 4	13	4.6	2	0.7	4	1.4	7	2.5	1	0.4	6	2.1	—	—
Lac St. Louis Site 5	22	5.6	2	0.5	3	0.8	16	4.1	1	0.3	6	1.5	1	0.3
Lac St. Louis Site 6	14	4.3	3	0.9	6	1.9	10	3.1	2	0.6	4	1.2	1	0.3
Lac St. Louis Site 7	6	4.2	—	—	—	—	5	3.5	1	0.7	1	0.7	1	0.7
Lac St. Louis Site 8	13	5.6	1	0.4	3	1.3	14	6.0	2	0.9	5	2.1	1	0.4
Lac St. Louis Site 9	2	6.7	—	—	1	3.3	1	3.3	—	—	—	—	—	—
LaPrairie basin Site 3	2	18.2	1	9.1	2	18.2	1	9.1	—	—	—	—	—	—
LaPrairie basin Site 7a	1	25.0	—	—	—	—	—	—	—	—	—	—	—	—
LaPrairie basin Site 13a	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Southern Indian Lake-Notigi Reservoir														
Wupaw Bay basin	—	—	—	—	—	—	1	3.8	—	—	—	—	—	—
South Bay basin	—	—	—	—	—	—	1	1.5	1	1.5	—	—	—	—
Mynarski Lake basin	3	3.9	2	2.6	—	—	—	—	—	—	1	1.3	1	1.3
Rat Lake basin (spring)	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rat Lake basin (summer)	—	—	1	9.1*	—	—	—	—	—	—	—	—	—	—
Last Mountain Lake Site I	5	0.8	1	0.2	1	0.2	—	—	—	—	2	0.3	—	—
Last Mountain Lake Site II	—	—	—	—	—	—	1	1.1	1	1.1	1	1.1	—	—
Tobin Lake Site 2	7	2.0	2	0.6	—	—	4	1.2	1	0.3	3	0.9	—	—
Tobin Lake Site 17	—	—	1	1.10	—	—	1	1.10	—	—	—	—	—	—
Port Hope Harbour (Sites B,C,E)	3	4.5	4	6.1	2	3.0	—	—	1	1.5	3	4.5	—	—

*Single larval specimen.

from the factory complex at Beauharnois, Quebec. Most of those larvae with double deformities were also from Lac St. Louis or the Laprairie basin sites. The only other sites to have double deformities were Port Hope Harbour and Tobin Lake Site 2, both of which are seriously contaminated.

Discussion

A number of authors (Hamilton and Saether 1971; Hare and Carter 1976; Warwick 1985, 1988; Wiederholm 1984; van Urk et al. 1985) have observed that different chironomid genera display different levels of tolerance or susceptibility to contaminants. In his survey of Swedish lakes, Wiederholm (1984) noted that deformed mouthparts occurred in several species, including species which were not closely related or which otherwise differed in their mode of living. As examples, he noted that deformities had been found both in the sedentary, filter-feeding larvae of *Chironomus* and in the predatory, free-living larvae of *Procladius*. Van Urk et al. (1985) found few deformed *Procladius* larvae in areas where numerous deformed *Chironomus* coexisted. They wondered whether *Procladius* larvae were less sensitive to pollutants or whether their lack of (morphological) response was due to their different food requirements. Warwick (1985) also noted the difference in response between *Chironomus* and *Procladius*. He suggested that the greater response in *Chironomus* might be attributed to the genus feeding directly on contaminant-laden organic detritus.

The difference in response between *Chironomus* and *Procladius* is apparent in Table 6, particularly in sites like Tobin Lake and Port Hope Harbour where there is some balance between the proportions of the two genera. In the Tobin Lake sites, the frequency of deformities is 10–15× greater in *Chironomus* than in *Procladius*. In Port Hope Harbour, the results are similar, but the differential (5×) is not quite as great.

This relationship seems to hold regardless of the proportions formed between *Chironomus* and *Procladius*. In Mynarski Lake and Last Mountain Lake Site II, where the proportions of *Chironomus* and *Procladius* are very similar, the frequency of deformities is 2–3× greater in *Chironomus*. In Rat Lake where *Chironomus* species make up almost the entire community in both spring and summer data sets, the frequency of deformities varied between 24 and 7× in favor of *Chironomus*. Because *Procladius* represents such a very minor component of these fauna, the data must be viewed with caution. Rare fauna are always difficult to treat, but this deficiency may be offset in part at least by the very large sample size ($n = 890$ and 1260, respectively) on which these data are based.

The relationship also holds when *Procladius* is the dominant component of the fauna. In Last Mountain Lake Site I where *Procladius* forms more than 50% of the fauna, the frequency of deformities in *Chironomus* is 11× higher than in *Procladius*. In Wupaw Bay and South Bay where few or no *Chironomus* were found, the frequency of deformities was ~3–4%.

It seems clear from these data that *Chironomus* is more sensitive to contaminant stress than *Procladius*. By what mecha-

TABLE 5. Frequency (%) of single and multiple deformities in *Procladius* spp. larvae; in terms of the *Procladius* component of the chironomid community.

	Single		Double		Triple	
	No.	Percent	No.	Percent	No.	Percent
St. Lawrence River						
Lac St. Louis Site 1	1	5.3	1	5.3	—	—
Lac St. Louis Site 2	18	9.5	1	0.5	—	—
Lac St. Louis Site 3	6	11.5	—	—	—	—
Lac St. Louis Site 4	30	10.7	—	—	1	0.4
Lac St. Louis Site 5	43	11.0	3	0.8	1	0.3
Lac St. Louis Site 6	31	9.6	3	0.9	1	0.3
Lac St. Louis Site 7	12	8.5	1	0.7	—	—
Lac St. Louis Site 8	31	13.2	4	1.7	—	—
Lac St. Louis Site 9	4	13.3	—	—	—	—
LaPrairie basin Site 3	4	36.4	1	9.1	—	—
LaPrairie basin Site 7a	1	25.0	—	—	—	—
LaPrairie basin Site 13a	—	—	—	—	—	—
Southern Indian Lake-Notigi Reservoir						
Wupaw Bay basin	1	3.8	—	—	—	—
South Bay basin	2	3.1	—	—	—	—
Mynarski Lake basin	7	9.2	—	—	—	—
Rat Lake basin (spring)	—	—	—	—	—	—
Rat Lake basin (summer)	1	9.1	—	—	—	—
Last Mountain Lake Site I	9	1.5	—	—	—	—
Last Mountain Lake Site II	3	3.2	—	—	—	—
Tobin Lake Site 2	11	3.2	3	0.9	—	—
Tobin Lake Site 17	2	2.1	—	—	—	—
Port Hope Harbour (Sites B,C,E)	11	16.7	1	1.5	—	—

TABLE 6. Comparison of the frequency of deformities in *Chironomus* and *Procladius* spp. larvae.

	Percent *Chironomus* spp.	Percent *Chironomus* spp. deformed	Percent *Procladius* spp.	Percent *Procladius* spp. deformed
St. Lawrence River				
Lac St. Louis Site 1	2.2	100.0	41.3	10.5
Lac St. Louis Site 2	0.0	0.0	75.1	10.0
Lac St. Louis Site 3	0.0	0.0	73.2	11.5
Lac St. Louis Site 4	0.3	0.0	76.4	11.0
Lac St. Louis Site 5	0.4	0.0	79.5	12.0
Lac St. Louis Site 6	3.4	6.7	73.5	10.8
Lac St. Louis Site 7	0.3	0.0	38.5	9.2
Lac St. Louis Site 8	5.5	2.6	33.2	15.0
Lac St. Louis Site 9	0.0	0.0	63.8	13.3
LaPrairie basin Site 3	58.1	36.1	17.7	45.5
LaPrairie basin Site 7a	58.5	60.0	23.5	25.0
LaPrairie basin Site 13a	30.0	66.7	10.0	0.0*
Southern Indian Lake-Notigi Reservoir				
Wupaw Bay basin	0.6	0.0	7.6	3.8
South Bay basin	0.0	0.0	41.7	3.1
Mynarski Lake basin	84.9	19.9	10.4	9.2
Rat Lake basin (spring)	98.9	24.0	0.7	0.0
Rat Lake basin (summer)	97.0	65.1	0.9	9.1*
Last Mountain Lake Site I	22.1	16.2	52.7	1.5
Last Mountain Lake Site II	89.5	11.0	9.9	3.2
Tobin Lake Site 2	48.2	41.3	34.1	4.1
Tobin Lake Site 17	49.8	31.8	31.4	2.1
Port Hope Harbour (Sites B,C,E)	23.0	87.5	37.9	18.2

*Single larval specimens.

nism *Procladius* is more tolerant is not known. Morphologically, *Procladius* seems to be a simpler animal than *Chironomus* and has fewer responding structures, but it is unlikely that this in itself can account for the differences observed in frequency of response. It seems likely that these are based more on physiological differences than simply on morphological differences.

These apparent differences in tolerance pose an interesting scenario for the Lac St. Louis sites. *Chironomous* and *Procladius* usually co-exist to some degree in moderately oligotrophic to mesotrophic environments, but in this case, except for a single fourth instar larva, the only *Chironomus* larvae found at Sites 1, 6, and 8 were first instar larvae of *Chironomus plumosus*. No *Chironomus* larvae were found in the other five sites in Lac St. Louis. In addition, the frequency of deformities within the *Procladius* community are 2–4× higher than either Wupaw Bay or South Bay, those sites most directly comparable with the Lac St. Louis sites. If *Procladius* is indeed the more tolerant of the two genera, it may be that *Chironomus* is on the verge of extinction in these areas of the St. Lawrence River.

Saether (1970) documented much the same faunal imbalance in Skaha Lake, British Columbia, where *Procladius* was the only remaining member of the deeper-water mesotrophic community. Since the oxygen deficit was not severe enough to eliminate species of *Chironomus*, he suggested that the deeper-water community had very recently been eliminated by deteriorating trophic conditions and for some reason had not as yet been replaced by a more eutrophic fauna. He suggested that high concentrations of insecticides from surrounding orchards were preventing normal community succession. Although his emphasis was different, what Saether seems to have documented was the elimination of a deeper-water community by contaminants rather than changes in community succession brought about by trophic change. In Lac St. Louis, oxygen depletion was not a determining factor either. If contaminants have eliminated *Chironomus* and their attendant fauna as both cases suggest, the importance of contaminants in modifying the processes of normal community succession and/or destroying benthic communities entirely can not be underestimated.

The data from the Laprairie basin suggest that, where oxygen conditions do become limiting, *Chironomus* may have an advantage over *Procladius*. Although no actual measurements were taken, the oxygen demand created by the raw sewage entering the basin from the Laprairie sewage treatment facility lead to seriously reduced oxygen levels toward Site 13a. The frequency of deformities in *Procladius* declined along this gradient, but in *Chironomus* they actually increased as conditions deteriorated. This same phenomenon was observed between the western and eastern basins of Pasqua Lake (Warwick 1980a). In this study, the frequency of deformities in *Chironomus* increased from 2.5 to 14.3% while those in *Procladius* declined from 2.1 to 0%. The concentrations of dissolved oxygen in the bottom waters of the two basins was 2.3–3.4 mg $O_2 \cdot L^{-1}$ and 0 mg $O_2 \cdot L^{-1}$, respectively. *Chironomus* is particularly adapted to low oxygen concentrations (Nagell and Landahl 1978) and its haemoglobin (Pinder 1986) may give this genus the advantage over *Procladius* under these circumstances.

The interplay between mortality and morphological response seems to represent another aspect of the phenomenon of quantal dose–response. Warwick (1985) first noted this phenomenon in the larval antennae. In this structure, he noted that morphological response first increased as contaminant concentrations increased, but then declined to the point that in very highly contaminated environments like Port Hope Harbour, deformed antennae were no longer a feature of an otherwise heavily deformed *Chironomus* fauna. In the case of the Laprairie basin, it may be that we are witnessing the same phenomenon, but instead of a single structure, the community itself can no longer respond and is on the verge of local extinction.

The fact that these two genera differ in their tolerance or susceptibility to contaminants is important from the point of view of measuring the impact of contaminants and/or assessing the health of aquatic ecosystems. Up until now, the system being developed has been based solely on the genus *Chironomus*. Now, the fact that *Procladius* overlaps and begins to respond significantly beyond the levels at which *Chironomus* can survive, means that the tool can be extended to cover a greater range of contaminant concentration. And if species more sensitive than *Chironomus* can be isolated, the sensitivity of the tool possibly may be extended into the lower concentration ranges and certainly into different trophic environments. This goal probably is achievable because Hamilton and Saether (1971) have already described deformities in genera like *Stictochironomus* and *Protanypus*. Both are important indicators of oligotrophic conditions under the lake classification system. It may also be possible to fit other, more sensitive members of the benthic community into such a scheme to provide even broader monitoring coverage.

Acknowledgements

I gratefully acknowledge the field support provided by H. H. Sloterdijk and staff, Water Quality Branch, Montreal, in collecting the St. Lawrence River material. Reviews and comments were provided by K. Gordon, C. S. L. Ommanney, and J. Gilliland. Funding was provided by the National Hydrology Research Institute.

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Journal canadien des sciences halieutiques et aquatiques

Volume 46, No. 7, July 1989

Volume 46, nº 7, juillet 1989

Irvine, J. R., and B. R. Ward. Patterns of timing and size of wild coho salmon (*Oncorhynchus kisutch*) smolts migrating from the Keogh River watershed on northern Vancouver Island 1086–1094

Likely, C. G., and M. D. B. Burt. Cultivation of *Pseudoterranova decipiens* (sealworm) from third-stage larvae to egg-laying adults in vitro 1095–1096

Stout, R. J. Effects of condensed tannins on leaf processing in mid-latitude and tropical streams: a theoretical approach 1097–1106

Hard, J. J., A. C. Wertheimer, and W. F. Johnson. Geographic variation in the occurrence of red- and white-fleshed chinook salmon (*Oncorhynchus tshawytscha*) in western North America 1107–1113

Waltman, W. D., E. B. Shotts, and R. E. Wooley. Development and transfer of plasmid-mediated antimicrobial resistance in *Edwardsiella ictaluri* 1114–1117

Gardner, W. S., B. J. Eadie, J. F. Chandler, C. C. Parrish, and J. M. Malczyk. Mass flux and "nutritional composition" of settling epilimnetic particles in Lake Michigan 1118–1124

Gauvin, J. M., W. S. Gardner, and M. A. Quigley. Effects of food removal on nutrient release rates and lipid content of Lake Michigan *Pontoporeia hoyi* 1125–1130

Bloom, N. Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection 1131–1140

Ankley, G. T., V. S. Blazer, S. M. Plakas, and R. E. Reinert. Dietary lipid as a factor modulating xenobiotic metabolism in channel catfish (*Ictalurus punctatus*) 1141–1146

Bigler, B. Mechanism and occurrence of focal scale resorption among chum salmon (*Oncorhynchus keta*) of the North Pacific Ocean 1147–1153

Mallet, A. L., and C. E. A. Carver. Growth, mortality, and secondary production in natural populations of the blue mussel, *Mytilus edulis* 1154–1159

Bacher, C. et P. Goulletquer. Comparaison des relations trophiques de *Ruditapes philippinarum* en milieux estuarien et océanique à partir d'un modèle de croissance 1160–1170

McCauley, E., J. A. Downing, and S. Watson. Sigmoid relationships between nutrients and chlorophyll among lakes 1171–1175

Prairie, Y. T., C. M. Duarte, and J. Kalff. Unifying nutrient–chlorophyll relationships in lakes 1176–1182

Peterman, R. M. Application of statistical power analysis to the Oregon coho salmon (*Oncorhynchus kisutch*) problem 1183–1187

Holtze, K. E., and N. J. Hutchinson. Lethality of low pH and Al to early life stages of six fish species inhabiting PreCambrian Shield waters in Ontario 1188–1202

Bates, S. S., C. J. Bird, A. S. W. deFreitas, R. Foxall, M. W. Gilgan, L. A. Hanic, G. E. Johnson, A. W. McCulloch, P. Odense, R. Pocklington, M. A. Quilliam, P. G. Sim, J. C. Smith, D. V. Subba Rao, E. C. D. Todd, J. A. Walter, and J. L. C. Wright. Pennate diatom *Nitzschia pungens* as the primary source of domoic acid, a toxin in shellfish from eastern Prince Edward Island, Canada 1203–1215

Mathews, S. B., and Y. Ishida. Survival, ocean growth, and ocean distribution of differentially timed releases of hatchery coho salmon (*Oncorhynchus kisutch*) 1216–1226

Cooper, W. J., D. R. S. Lean, and J. Carey. Spatial and temporal patterns of hydrogen peroxide in lake waters 1227–1231

Canfield, D. E. Jr., E. Phlips, and C. M. Duarte. Factors influencing the abundance of blue-green algae in Florida lakes 1232–1237

Simard, Y., and D. L. Mackas. Mesoscale aggregations of euphausiid sound scattering layers on the continental shelf of Vancouver Island 1238–1249

Ehlinger, T. J. Foraging mode switches in the golden shiner (*Notemigonus crysoleucas*) 1250–1254

Warwick, W. F. Morphological deformities in larvae of *Procladius* skuse (Diptera:chironomidae) and their biomonitoring potential 1255–1270

Announcement/Avis 1271

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Ministre des Approvisionnements et Services Canada 1989

CJFSDX46(7) 1086–1271 (1989)
ISSN 0706-652X

Subscription rates/Abonnements

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